Improving antimicrobial use and control of resistant micro-organisms in the hospital

Ina Willemsen

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Improving antimicrobial use and control of resistant micro-organisms in the hospital
promotoren: prof.dr. J.A.J.W. Kluytmans
prof.dr. P.H.M. Savelkoul
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General Introduction and outline of the thesis
GENERAL INTRODUCTION

In 1945 Sir Alexander Fleming was the first to warn for the threat of antimicrobial resistance in his Nobel Lecture on the discovery of the antimicrobial effect of penicillin [1]. Many antimicrobial agents have been developed since. Whenever a new agent was introduced into clinical practice, however, resistance to the drug appeared sooner or later [2]. This has resulted in a situation where more and more infections occur that are hard to treat. Experts fear that a post-antibiotic era may be reality in the near future [3]. Also antimicrobial resistance results in the use of more expensive agents and in an increase in morbidity and mortality. This contributes substantially to the rising health care costs [4-6].

On November fifth 2009, President Barack Obama and the Swedish Prime Minister Reinfeldt, representing the European Union Presidency, joined forces to address the urgency of the ongoing problem of antimicrobial resistance and the need for solutions by signing an agreement. The World Health Organization has also identified antimicrobial resistance as one of the greatest threats to human health [7].

It is clear that antibiotic use is associated with the development of resistance. The general principle of this association is often expressed in a simple verb: “The more you use it, the sooner you lose it” [8]. This makes antimicrobials different from all other classes of therapeutic agents. Other therapeutic agents only affect patients where antimicrobials has impact on both patient and bacteria. This can affect patients other than the patient who is treated, namely the development of resistance that can spread to others [9]. This is called patient dependency. Although the patient being treated may receive an effective antibiotic, this may still create a problem for other patients in the future.

To limit the development of resistance and thereby ensure treatment options for future patients, it is important to restrictively use antimicrobials that are currently available. This means selecting the most effective agent with a maximal clinical therapeutic effect and minimal side effects. These include both drug-related toxicity and the development of antimicrobial resistance [7].

In the Netherlands the use of antimicrobial agents for human health is much lower than in the surrounding countries [10,11]. Therefore, the problem of resistance in the Netherlands has been relatively limited. However, the use of antibiotics and the associated development of resistance is increasing and becoming problematic, especially in hospitals [12,13]. In hospitals the use of antibiotics is relatively high, which promotes the selection of resistant populations that may have been present on admission [14]. Also, nosocomial transmission between patients may occur, either directly or indirectly, i.e. through healthcare workers or instruments [15].
To control and limit antimicrobial resistance and the transmission of resistant micro-organisms in hospitals it is important to have both a restrictive antimicrobial policy and an adequate infection control policy. Information about the appropriateness of use of antimicrobial agents and transmission dynamics should be available in order to perform interventions on inappropriate use and to adapt the infection control policies to current situations.

**OUTLINE OF THE THESIS**

The purposes of the studies presented in this thesis is to develop a tool to measure the appropriateness of Antimicrobial Therapy (AMT), to determine the role of nosocomial transmission in the epidemiology of resistant micro-organisms, to improve the appropriateness of AMT by implementing targeted interventions and to measure the effect of these interventions on the observed antimicrobial resistance.

**Chapter 2** describes a method to measure and judge the use of antimicrobials on a patient level. Prevalence surveys were used as an additional tool to the more common method of expressing the use in Defined Daily Doses (DDD) per patient days or admissions.

**Chapter 2.1** focuses on the usefulness of prevalence surveys to judge the appropriateness of AMT in a single hospital. Determinants for inappropriate use of AMT were determined by repeated prevalence surveys.

**Chapter 2.2** describes the surveillance of appropriateness of AMT in a point prevalence survey in 19 Dutch hospitals. Judgment of the appropriateness of AMT was assessed according to a standardised algorithm based on the local antimicrobial prescription guidelines. Furthermore, the possibility of benchmarking between hospitals was investigated.

**Chapter 3** focuses on the incidence and spread of antimicrobial resistance in the hospital. Different modes of spread of antimicrobial resistance were studied: transmission of (complete) bacteria, but also horizontal gene transfer. Additionally, the role of the use of AMT and infection control measures was assessed.

**Chapter 3.1** describes the dynamics of antimicrobial use and resistance in separated medical wards within one hospital.

**Chapter 3.2** presents the contribution of horizontal spread to the incidence of highly resistant micro-organisms in an endemic setting and the importance of the Intensive Care Unit as determinant for antimicrobial resistance.

**Chapter 3.3** describes the role of integrons in different species of highly resistant Gram negative micro-organisms and the contribution of horizontal gene transfer to the spread of resistance in a teaching hospital.
Chapter 3.4 is devoted to the TRIANGLE study (TRIANGLE, Testing Resistance in relation with Infection Control and Antimicrobial Use in the Netherlands, Getting Less Problems by working together), a multi-center study in which the incidence, and transmission, of highly resistant Gram negative rods is determined in different university-, teaching- and non-teaching hospitals. Furthermore, the relation between resistant micro-organisms and infection control practices and antimicrobial use are investigated.

Chapter 4 focuses on targeted interventions aimed on the improvement of antimicrobial use.

Chapter 4.1 describes the implementation of a protocol for perioperative antimicrobial prophylaxis. In this study the effect of simplification and standardisation of different protocols on the timing of administration of antimicrobial prophylaxis was investigated.

Chapter 4.2 shows the effects of targeted interventions aimed at the use of fluoroquinolones and the subsequent effect on the observed fluoroquinolone resistance.

Finally, in chapter 5 the main findings of the studies in this thesis are discussed and recommendations for future research are given.
REFERENCES

Measuring Antimicrobial Use on a Patient Level
2.1

Appropriateness of Antimicrobial Therapy Measured by Repeated Prevalence Surveys

Ina Willemsen\textsuperscript{1}, Anneke Groenhuijzen\textsuperscript{2}, Diana Bogaers\textsuperscript{1}, Arie Stuurman\textsuperscript{3}, Peter van Keulen\textsuperscript{1}, Jan Kluytmans\textsuperscript{1,4}

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ABSTRACT

Objective
The objective of this study was to determine if prevalence surveys are useful tools to determine the appropriateness of antimicrobial therapy (AMT) and determinants of inappropriate AMT.

Methods
The study was performed in a 1,350-bed teaching hospital including all medical specialties. Six consecutive 1-day prevalence surveys of in-patients were performed twice yearly from 2001 to 2004. Data on the demographics, infections, and AMT were gathered. The appropriateness of AMT was assessed according to a standardised algorithm based on the local AMT prescription guidelines.

Results
On average, 684 patients were included in each survey (total, 4,105). The use of AMT as determined in the prevalence survey corresponded to the annual data from the pharmacy department. Nine hundred thirty-eight (22.9%) of the patients received AMT, and in 351 (37.4%) of these patients AMT was inappropriate. Only 25 (0.6%) patients did not receive AMT, although it was indicated. After multivariate analysis, the use of fluoroquinolones was the only statistically significant variable associated with inappropriate use.

Conclusions
Prevalence surveys proved to be useful tools to judge the appropriateness of AMT and to identify determinants of inappropriate use. This study showed that in a setting with a low use of AMT, there were few patients who inadvertently do not receive AMT. On the other hand, a substantial number of the patients were treated inappropriate.
INTRODUCTION

Resistance to antimicrobial drugs is a serious and increasing problem throughout the world [1,2]. Hospitals play a key role in the development of antimicrobial resistance. To control the development of resistance, a restrictive antimicrobial policy in combination with effective infection control measures to prevent the spread of resistant micro-organisms is advisable. Therefore, local or national guidelines for antimicrobial therapy (AMT) have been developed [3-6].

The implementation of these guidelines and their effectiveness is questionable regarding the ever-increasing problem of resistance. More insight into the actual implementation of the prescription guidelines is needed.

Investigating the consumption of antimicrobial agents from the pharmacy department is often used and provides an estimate of the total use AMT. Using this method, it is clear that huge variations exist between countries and between hospitals within countries [7]. However, it does not provide insight into the appropriateness of AMT and about determinants of inappropriate use.

The objectives of this study were to determine the usefulness of prevalence surveys to measure antimicrobial consumption in the hospital, to determine the appropriateness of AMT, and to identify determinants of inappropriate use.

PATIENTS AND METHODS

Setting

The Amphia hospital is a 1,350-bed teaching hospital with three locations. All medical specialties are available. In 2004 there were 39,704 admissions and 273,125 bed days. The average length of stay was 6.9 days.

Prevalence surveys

Prevalence surveys are performed twice a year, in the spring and in the autumn. All patients that are present in the hospital at 6 a.m. on the day of the survey were included. Patients in day care, in psychiatric wards, or on hemodialysis were excluded.

Infection control practitioners (ICP) collected the data from the medical and nursing records and by conversation with the nursing staff. All data were registered using standardised forms. There were six ICPs participating, who were all trained in national surveillance workshops to gather the data in a standardised way.
From each patient the following demographic variables were recorded: age, sex, medical specialty, medical ward, and presence of infection on admission. Nosocomial infections were recorded using the Centers for Disease Control and Prevention definitions [8,9], as is whether patients were still symptomatic or were still being treated on the day of the survey. Judgment of the infection data (infection on admission and kind of nosocomial infection) was performed by the ICPs.

Furthermore, the use of antibiotics and variables like dose-related issues were noted. The pharmacy dispensing data were not validated on a patient level and therefore not suitable for this purpose. If more than one antibiotic was prescribed for one patient, all antibiotics, with a maximum of three, were registered. Antifungal and antiviral therapy as well as medication for tuberculosis was excluded from the study.

**Appropriateness of antimicrobial therapy**

The appropriateness of AMT was determined using a standardised method developed by Gyssens et al. [10]. The following classifications were used: correct decision, incorrect decision, incorrect choice, incorrect use, or insufficient data. This score system only takes into account patients that are on AMT. Using prevalence surveys it is also possible to examine the appropriateness of not receiving AMT.

Antibiotic use categorised as “correct decision” was deemed appropriate. Antibiotic use categorised as “incorrect decision,” “incorrect choice,” or “incorrect use” was deemed inappropriate. The criteria for evaluation are summarised in Table 1.

The use of antibiotics was judged according to the local AMT prescription guidelines. The local AMT prescription guidelines was written by a local team of consultant microbiologists, infectious disease physicians, and pharmacists based on national and international guidelines adapted to the local susceptibility patterns of pathogens. All medical specialists working in the hospital were invited to comment on a draft version, and finally the local committee on antimicrobial therapy sanctions these guidelines.

The hospital pharmacist performed the first screening of the appropriateness of AMT, while more complicated cases were judged by a consultant microbiologist. Complicated cases included all patients in Intensive Care, patients who received antibiotics without having an active infection, patients who did not receive antibiotics and did have an active infection, patients who received an antibiotic that was not indicated by the local AMT prescription guidelines, and all cases that were considered questionable by the person who performed the initial screening (hospital pharmacist or study coordinator).

**General data on antimicrobial use**

The annual data on antimicrobial use from the pharmacy department were used to validate the observations in the prevalence surveys (the annual consumption data of antibiotic use from the pharmacy department have been validated since 2002). In addition, the number of admissions
and the average length of stay were obtained from the hospital administration. The antibiotic consumption was calculated to defined daily doses (DDD)/100 patient days according to the ATC/DDD index 2005 from the WHO Collaborating Centre for Drug statistics Methodology [11].

Data analyses, quality control, and statistics

Privacy of patients was provided by coding all data according to the requirements of the privacy regulation in the Amphia hospital. The data were entered in a database, double checked by the investigator and ICP of the project, and analysed using the Statistical Package for Social Sciences software (SPSS, version 12.0). Before as well as during the project, the case-finding methods and interpretation of the medical information by the ICP were validated for intra- and interobserver reproducibility by discussing all nosocomial infections with another ICP, and if they disagree the case was resolved by plenary discussion. Furthermore, the ICP and the consultant microbiologist discussed all completed forms from ICU patients.

Categorical variables were analysed by Fisher’s exact test or the chi-square test when appropriate, and continuous variables were analysed using a t test or Mann-Whitney U test when appropriate. Trends over time were examined using linear regression analysis. Binary logistic regression analysis was performed to control for confounding. All variables with a $p$ value below 0.1 were entered into the model. Statistical significance was accepted when the chance for coincidence was less than 5%.

### Table 1: Score system for the appropriateness of antimicrobial therapy (AMT)

<table>
<thead>
<tr>
<th>Action and score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correct decision</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No AMT; no infection; no AMT needed</td>
</tr>
<tr>
<td>2</td>
<td>No AMT; infection; no AMT needed</td>
</tr>
<tr>
<td>3</td>
<td>AMT; infection; APP choice; AP use</td>
</tr>
<tr>
<td><strong>Incorrect decision</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No AMT; infection; AMT needed</td>
</tr>
<tr>
<td>2</td>
<td>AMT; no infection; no prophylaxis; no AMT needed</td>
</tr>
<tr>
<td>3</td>
<td>AMT; no infection; prophylaxis; no AMT needed</td>
</tr>
<tr>
<td><strong>Incorrect choice</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Divergence from guideline</td>
</tr>
<tr>
<td><strong>Incorrect use</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>IAAP dosage</td>
</tr>
<tr>
<td>2</td>
<td>IA timing</td>
</tr>
<tr>
<td>3</td>
<td>IA administration</td>
</tr>
<tr>
<td>4</td>
<td>IA duration of therapy</td>
</tr>
<tr>
<td><strong>Missing data</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No AMT; not enough diagnostic information about infection</td>
</tr>
<tr>
<td>2</td>
<td>Infection; not enough diagnostic information if AMT is needed</td>
</tr>
<tr>
<td>3</td>
<td>AMT; not enough diagnostic information about infection</td>
</tr>
<tr>
<td>4</td>
<td>Infection; not enough information about AMT</td>
</tr>
</tbody>
</table>

*a* AP: Appropriate  
*b* IA: Inappropriate
RESULTS

Demographics and infections
Between 2001 and 2004, six surveys were performed, and a total of 4,105 patients were included. Overall, 1,894 (46.1%) were male, and the mean age was 59.9 years (standard deviation, 22.7); both variables were constant over time. An infection on admission was present in 685 patients (16.7%), and 359 patients (8.7%) had at least one active nosocomial infection on the day of the survey. Figure 1 shows the trends over time of infection on admission and nosocomial infections. There was a significant increase in the number of patients with an infection on admission in the hospital ($p=0.02$) and in the overall proportion of patients with nosocomial infections ($p=0.03$).

![Figure 1: Trends over time of infections on admission and nosocomial infections in six surveys between 2001 and 2004](image)

Antimicrobial therapy by prevalence surveys
A total of 938 patients (22.9%) were on AMT. Of those 938 patients, 48 (5.1%) were treated with two antibiotics, and 10 (1.1%) were treated with three antibiotics. The prevalence of AMT was consistent over time, and no significant trend was observed.

Antimicrobial therapy by pharmacy department
The PDD/100 patient days increased from 22.5 in 2002 to 26.5 in 2003 to 29.5 in 2004 (corresponding with a DDD/100 patient day from 32.1 in 2002, 37.7 in 2003, and 42.6 in 2004).

Appropriateness of AMT
In 351 (37.4%) patients of the total of 938 who were on AMT, AMT was deemed inappropriate. More specifically, in 123 patients (13.0%) AMT was unjustified, in 140 patients (14.9%) an incor-
rect choice was made, and in 88 patients (9.4%) the correct antibiotic was used but it was used incorrectly.

There were no significant differences in the appropriateness of AMT between the six surveys, and there was no significant trend over time (Figure 2). Twenty-five patients (0.6%) did not receive AMT, although this was indicated. Finally, 71 (1.7%) patients could not be judged because of insufficient information.

**Determinants of inappropriate use of antibiotics**

In the univariate analysis, the use of fluoroquinolones and co-amoxiclavulanic acid were statistically significantly associated with more frequent inappropriate use of AMT (Table 2). The use of cephalosporines, penicillins, carbapenem, metronidazole, and rifampicin were significantly associated with more frequent appropriate use of AMT (Table 2).

Considering the use of AMT in the different medical specialties, urology; ear, nose, and throat; geriatrics; and neurology proved to be statistically significantly associated with more frequent inappropriate use, and pediatrics was statistically significantly associated with more frequent appropriate use (Table 3).

Other factors that were statistically significantly associated with more appropriate use were younger age and the presence of an infection on admission (Table 4). After multivariate analysis, the use of fluoroquinolones was the only statistically significant factor associated with inappropriate use.
Table 2: The appropriateness of antimicrobial therapy in different groups of antibiotics

<table>
<thead>
<tr>
<th>Antimicrobial agent(s)</th>
<th>AP(^a) use (n)</th>
<th>IA(^b) use (n)</th>
<th>% of total use</th>
<th>RR(^c) for IA use (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>penicillins(^c)</td>
<td>117</td>
<td>37</td>
<td>15.1</td>
<td>0.55 (0.41 - 0.74)</td>
</tr>
<tr>
<td>co-amoxicillin-clavulanic acid</td>
<td>181</td>
<td>158</td>
<td>33.2</td>
<td>1.30 (1.11 - 1.53)</td>
</tr>
<tr>
<td>1(^{st}) &amp; 2(^{nd}) generation cephalosporins</td>
<td>85</td>
<td>30</td>
<td>11.3</td>
<td>0.62 (0.45 - 0.85)</td>
</tr>
<tr>
<td>3(^{rd}) &amp; 4(^{th}) generation cephalosporins</td>
<td>38</td>
<td>14</td>
<td>5.1</td>
<td>0.66 (0.42 - 1.04)</td>
</tr>
<tr>
<td>co-piperacillin-tazobactam</td>
<td>6</td>
<td>1</td>
<td>0.7</td>
<td>0.36 (0.06 - 2.19)</td>
</tr>
<tr>
<td>carbapenems</td>
<td>12</td>
<td>0</td>
<td>1.2</td>
<td>N.A.(^d) (p=0.005)</td>
</tr>
<tr>
<td>aminoglycosides</td>
<td>14</td>
<td>6</td>
<td>2.0</td>
<td>0.75 (0.38 - 1.46)</td>
</tr>
<tr>
<td>quinolones</td>
<td>42</td>
<td>71</td>
<td>11.1</td>
<td>1.72 (1.45 - 2.03)</td>
</tr>
<tr>
<td>trimethoprim-sulfamethoxazole</td>
<td>30</td>
<td>21</td>
<td>5.0</td>
<td>1.03 (0.74 - 1.45)</td>
</tr>
<tr>
<td>lincosamides / macrolides</td>
<td>29</td>
<td>16</td>
<td>4.4</td>
<td>0.88 (0.59 - 1.32)</td>
</tr>
<tr>
<td>metronidazole</td>
<td>44</td>
<td>14</td>
<td>5.7</td>
<td>0.59 (0.37 - 0.93)</td>
</tr>
<tr>
<td>vancomycin</td>
<td>7</td>
<td>4</td>
<td>1.1</td>
<td>0.91 (0.41 - 2.00)</td>
</tr>
<tr>
<td>tetracyclines</td>
<td>7</td>
<td>6</td>
<td>1.3</td>
<td>1.16 (0.64 - 2.09)</td>
</tr>
<tr>
<td>nitrofurantoin</td>
<td>7</td>
<td>9</td>
<td>1.6</td>
<td>1.42 (0.91 - 2.20)</td>
</tr>
<tr>
<td>rifampicin</td>
<td>14</td>
<td>0</td>
<td>1.4</td>
<td>N.A.(^d) (p=0.001)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>633</td>
<td>387</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) AP: appropriate; \(^b\) IA: inappropriate; \(^c\) RR: relative Risk; 
\(^d\) NA: not applicable. In these cases a p-value is given

# 60 patients were not included in this table because of insufficient information, and 58 patients were treated with more than one antibiotic.

Table 3: The Appropriateness of Antimicrobial Therapy by Medical Specialty

<table>
<thead>
<tr>
<th>Medical speciality (n)</th>
<th>AP(^a) use (n)</th>
<th>IA(^b) use (n)</th>
<th>% of total use</th>
<th>RR(^c) for IA use (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>114</td>
<td>76</td>
<td>21.6</td>
<td>1.00 (0.82 - 1.22)</td>
</tr>
<tr>
<td>Internal medicine</td>
<td>97</td>
<td>74</td>
<td>19.5</td>
<td>1.11 (0.91 - 1.34)</td>
</tr>
<tr>
<td>Lung diseases</td>
<td>104</td>
<td>55</td>
<td>18.1</td>
<td>0.84 (0.67 - 1.06)</td>
</tr>
<tr>
<td>Orthopaedics</td>
<td>85</td>
<td>51</td>
<td>15.5</td>
<td>0.93 (0.73 - 1.17)</td>
</tr>
<tr>
<td>Cardiology</td>
<td>34</td>
<td>18</td>
<td>5.9</td>
<td>0.86 (0.59 - 1.26)</td>
</tr>
<tr>
<td>Paediatrics</td>
<td>35</td>
<td>10</td>
<td>5.1</td>
<td>0.54 (0.31 - 0.94)</td>
</tr>
<tr>
<td>Neurology</td>
<td>18</td>
<td>21</td>
<td>4.4</td>
<td>1.37 (1.01 - 1.85)</td>
</tr>
<tr>
<td>Urology</td>
<td>11</td>
<td>21</td>
<td>3.6</td>
<td>1.68 (1.29 - 2.19)</td>
</tr>
<tr>
<td>Gynaecology</td>
<td>13</td>
<td>5</td>
<td>2.1</td>
<td>0.69 (0.33 - 1.46)</td>
</tr>
<tr>
<td>Geriatrics</td>
<td>4</td>
<td>10</td>
<td>1.6</td>
<td>1.81 (1.29 - 2.55)</td>
</tr>
<tr>
<td>Other specialities</td>
<td>12</td>
<td>10</td>
<td>2.5</td>
<td>1.14 (0.72 - 1.82)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>527</td>
<td>351</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) AP: Appropriate; \(^b\) IA: Inappropriate; \(^c\) RR: relative Risk

# 60 patients were not included, because of insufficient information.
DISCUSSION

The mean prevalence of AMT by prevalence surveys was 0.26. The prevalence of AMT was stable over time, and none of the point prevalence estimates differed significantly from any other. There were no significant differences between the annual data from the pharmacy department and the estimates from the separate prevalence surveys. Only a small fraction of the patients could not be judged (1.7%) due to insufficient information. Therefore, a single prevalence survey offers a reliable estimate on the current use of AMT.

However, this estimate by itself offers no advantage over the data from the pharmacy department, which is easier to acquire. The added value of prevalence surveys is the possibility to relate AMT to an individual patient. First, the appropriateness of AMT can be determined. Second, by collecting demographic variables and infection-related information, it provides the determinants of inappropriate use of AMT. Third, it provides an estimate of the proportion of patients that did not receive AMT while this was indicated. Finally, point prevalence surveys are efficient methods, which are performed relatively easily and rapidly. The added values of repeated prevalence surveys are to observe trends over time and the effects of interventions.

In our study, an infection on admission was present in 16.7% of the patients, and 8.7% had at least one nosocomial infection on the day of the survey. For both types of infections there was a slight but significant increase over time. It is possible that this reflects a true increase, but it can also be due to a better recognition of the infections by the ICP who performed the survey over time. The reported prevalence of nosocomial infections varies widely. In a large national prevalence survey in the United Kingdom and Republic of Ireland, the average prevalence was 9.0%. In teaching hospitals it was 11.2% [12].

The prevalence of AMT was 0.26. To judge this figure, it is important to realise that the Netherlands has among the lowest use of AMT in Europe [7]. A recent study by Filius [13] showed that the average use in Dutch hospitals was 55 DDD/100 patient days. The mean use in our hospital between 2002 and 2004 was 37 DDD/100 patient days, which is on the lower edge for Dutch hospitals. Still, 37.4% of all patients on AMT were treated inappropriately. In 13% of those, AMT

### Table 4: Appropriateness of use of antimicrobial therapy by age and presence of infection

<table>
<thead>
<tr>
<th>Age and infection status</th>
<th>AP(^a) use</th>
<th>IA(^b) use</th>
<th>p-value</th>
<th>RR(^c) for IA use (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (yrs)</td>
<td>60.3</td>
<td>64.3</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Infection on admission (n)</td>
<td>262</td>
<td>141</td>
<td>0.006</td>
<td>0.79 (0.67 - 0.94)</td>
</tr>
<tr>
<td>At least 1 nosocomial. Infection (n)</td>
<td>127</td>
<td>90</td>
<td>0.63</td>
<td>1.05 (0.87 - 1.26)</td>
</tr>
<tr>
<td>Total (n)</td>
<td>527</td>
<td>351</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) AP: Appropriate; \(^b\) IA: Inappropriate; \(^c\) RR: Relative Risk
was not indicated at all. The latter comprises 3.0% of the total group of patients and may seem relatively unimportant. However, this means that annually more than 8,000 days of unjustified AMT were given in our hospital.

As indicated, the total use of AMT in the Netherlands in general and in our hospital in particular is low. This could lead to a situation in which patients who need AMT are not treated. The prevalence surveys provide information on the clinical situation of the patient, including infection-related information. Therefore, it is possible to identify those patients who inadvertently did not receive AMT (0.6%).

Six of these patients were treated with AMT shortly after the day of the survey, and seven suffered from minor infections and were discharged within 1 week after the survey. Although AMT was indicated, their outcome seemed not adversely affected at discharge. Four of the remaining were deliberately not treated. It can be concluded that this situation of restrictive AMT is not accompanied by frequent abstinence of indicated treatment.

The use of fluoroquinolones especially proved to be an independent risk factor for inappropriate use of AMT in this study. Carbapenem, co-piperacillin-tazobactam, and vancomycin were used rarely, and the use was highly appropriate. These antibiotics are classified as restricted agents in our hospital, and the pharmacy and microbiology departments closely monitor their application.

After multivariate analysis, the use of fluoroquinolones was the only statistically significant factor associated with inappropriate use. The areas of the hospitals where fluoroquinolones were used most inappropriately were identified as well. When patients in orthopedic surgery, urology, or neurology were treated with fluoroquinolones, more than 75% of the time it was inappropriate.

There was a significant relationship between more appropriate use of AMT and the presence of an infection on admission. The presence of nosocomial infections was not associated with more appropriate use. This could indicate that physicians are more aware of the correct antibiotic choice for community-acquired infections than for nosocomial infections. Also, it could be that an infection on admission is judged more carefully than when it develops during hospitalisation [14, 15].

The results from prevalence surveys offer a possibility for targeted interventions in problem areas. Subsequently, repeated prevalence surveys can be used to measure the effect of the intervention. During the study, from 2001 to 2004 no interventions in antibiotic use were initiated. Interim data were not used to direct antimicrobial therapy.

After interpretation of the results of the study, several interventions for improvement of the use of antibiotics were started. The first intervention concerned the standardisation of the
drugs for perioperative prophylaxis. Before the intervention, eight different antibiotics were used for this purpose, and after the intervention only three were used (cefazolin, metronidazole, and clindamycin). This standardisation resulted in a significant improvement of the timing of prophylaxis and a cost reduction of at least €40,000 per year.

The second intervention aimed to improve the use of ciprofloxacin by switching from intravenous to oral administration as soon as possible. Six months after the start, the use of intravenous ciprofloxacin has been decreased more than 50%. This offers an annual saving of at least €65,000. A project to reduce the total use of ciprofloxacin will start soon. Repeated prevalence surveys will be used as a tool to measure the effects of the interventions. In conclusion, prevalence surveys offer an effective tool to improve the quality of AMT.

ACKNOWLEDGMENTS

We are indebted to the infection control practitioners Gonny Moen, Henk Coertjens, Karin van Dijk, Miranda van Rijen, and Yvonne Hendriks for the collection of the data and Anja Boele for assistance with the data files.
REFERENCES

Appropriateness of Antimicrobial Therapy: A multicenter Prevalence Survey

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Submitted
ABSTRACT

Objective
A multicenter prevalence survey was performed to determine the prevalence and appropriate-
ness of antimicrobial therapy (AMT) and to identify determinants for inappropriate AMT.

Methods
Prevalence surveys of in-patients were performed at three moments in 2008 and 2009. Demographic, infection related and AMT-related data were collected at the hospital wards. The appropriateness of AMT was assessed according to a standardised algorithm based on the local AMT prescription guidelines.

Results
In 19 hospitals, a total of 7,853 patients were included, of which 2,327 (29.6%) patients were on AMT (range hospitals: 20.8-39.5). In 371 patients (16% of patients on AMT) treatment was considered inappropriately. In 265 patients on AMT (11.4%) the appropriateness was not judged because of insufficient information. The percentage of patients without judgment varied considerably between the participating hospitals (range 1.3-36.2%). Appropriate use was significantly associated with being on the Intensive care Unit, the presence of a central venous catheter and when β-lactamase sensitive penicillin was given. The use of fluoroquinolons was associated with more frequent inappropriate use.

Conclusions
Using prevalence surveys a considerable variation in the total use of antimicrobials and appropriateness of use was determined. To improve the completeness and reliability of the obtained results an intensive training of the observers and medical staff in recording information is necessary. The major and only determinant for inappropriate use was the use of fluoroquinolons.
INTRODUCTION

Point prevalence surveys are useful methods to investigate healthcare related events, including antimicrobial use. The first report on antimicrobial use measured in prevalence surveys was published in 1983 [1]. More recently a European network has standardised a method for to determine the prevalence of antimicrobial therapy (AMT) in hospitals [2].

In a previous report, we showed that besides the prevalence of AMT, also the appropriateness of AMT for individual patients could be determined. In addition it was possible to identify determinants of inappropriate use of AMT in a teaching hospital [3].

The objective of the current study was to determine if the methodology could be used in other hospitals as well and potentially be used as a tool for benchmarking. The study was coordinated by the PREZIES-network, a cooperation between participating hospitals, the Dutch Institute for Healthcare Improvement CBO and the National Institute for Public Health and the Environment (RIVM).

PATIENTS AND METHODS

Prevalence surveys

Three prevalence surveys of hospitalised patients were performed in spring 2008, autumn 2008 and spring 2009. All patients that were present in the hospital at 0.01 AM on the day of the survey were included. Patients in day-care (including haemodialysis patients) and in psychiatric wards were excluded.

Data were collected by Infection Control Practitioners (ICP) at the hospital wards. A standardised case record form, including demographic, infection-related and AMT-related data, was used. The ICP were trained in workshops to collect the data. From each patient the following demographic variables were recorded: age, sex, medical speciality, medical ward and presence of infection on admission. Nosocomial infections were recorded using the CDC definitions and patients had to be symptomatic or were still being treated on the day of the survey [4,5].

Furthermore, the use of antimicrobial agents and dosage were noted. If more than one antimicrobial was prescribed for one patient, all antimicrobials, with a maximum of three, were registered. Antifungal- and antiviral therapy as well as medication for tuberculosis were excluded from the study.

Appropriateness of antimicrobial therapy

The appropriateness of AMT is determined using a standardised method developed by Gyssens et al [6]. The following classifications are used: correct decision, incorrect decision, incorrect choice or insufficient data. This score system only takes into account patients that are on AMT.
In addition, it is possible to examine the appropriateness of not prescribing AMT. This was done as described in our previous study [3]. Antimicrobial use categorised as ‘correct decision’ is deemed as appropriate. Antimicrobial therapy was categorised as ‘incorrect decision’ and ‘incorrect choice’ was considered inappropriate. The criteria for evaluation are summarised in Table 1. The use of AMT was judged according to the local AMT prescription guidelines, based on the National Guidelines from the Working Party on Antibiotic use (SWAB), which were present in all participating hospitals [7,8].

The ICP performed the first screening of the appropriateness of AMT, while more complicated cases were judged by a consultant microbiologist. In addition, the consultant microbiologist judged all ICU patients, all patients who received antimicrobials without having an active infection according to the prevalence survey, all patients who did not receive antimicrobials and did have an active infection and all patients who received an antimicrobial agent that was not included in the local AMT guidelines.

For further analyses a judgment on a patient level was obtained. If all antimicrobial agents were considered appropriate the judgment on a patient level was “appropriate”, when one antimicrobial agent was considered inappropriate, the judgment on a patient level was “inappropriate” and when one antimicrobial agent was not judged due to insufficient information the judgment on a patient level was also considered insufficient information.

### Data-analyses, quality control & statistics

Privacy of patients was provided by coding all data according to the requirements of the existing privacy regulations in The Netherlands. The data were entered in the PREZIES database or a hospital-owned database and subsequently coded and transferred to PREZIES.

| Table 1: Score system for the appropriateness of antimicrobial therapy (AMT) |
|---------------------------------|---------------------------------|
| **Action and score**            | **Description**                 |
| Correct decision                |                                 |
| 1                               | No AMT; no infection; no AMT needed |
| 2                               | No AMT; infection; no AMT needed  |
| 3                               | AMT; infection; appropriate choice; appropriate use |
| Incorrect decision              |                                 |
| 1                               | No AMT; infection; AMT needed     |
| 2                               | AMT; no infection; no prophylaxis; no AMT needed |
| 3                               | AMT; no infection; prophylaxis; no AMT needed |
| Incorrect choice                |                                 |
| 1                               | Divergence from guideline        |
| Missing data                    |                                 |
| 1                               | No AMT; not enough diagnostic information about infection |
| 2                               | Infection; not enough diagnostic information if AMT is needed |
| 3                               | AMT; not enough diagnostic information about infection |
| 4                               | Infection; not enough information about AMT |
Data were analysed using the Statistical Package for Social Sciences software (SPSS version 17.0). The assessment of appropriateness of therapy from patient who were not judged because of insufficient information were identified as missing values. Categorical variables were analysed by Fisher’s exact test or the Chi-square test when appropriate and continuous variables were analysed using a t-test or Mann-Whitney U test when appropriate. Binary logistic regression analysis was performed. All variables with a p-value below 0.1 in univariate analyses were entered in to the model. Statistical significance was accepted when the chance for coincidence was less than 5%.

RESULTS

A total of 7,853 patients were included, from 19 hospitals. Thirteen hospitals participated in one survey, 5 hospitals participated in 2 surveys, and 1 hospital participated in all 3 surveys. On average 302 patients were included per hospital per prevalence survey (range: 103-552; SD 149).

Patient characteristics and nosocomial infections

Overall 3,784 (48.2%) patients were male, and the mean age was 62.3 years (median 67). On the day or the survey 426 patients (5.4%) had at least one active nosocomial infection.

Antimicrobial therapy

A total of 2,327 patients (29.6%) were on AMT (range hospitals: 20.8 – 39.5) (Figure 1). Of those 2,327 patients, 433 (18.6%) were treated with two antimicrobials, and 58 (2.6%) were treated with 3 or more antimicrobials. From all administrated antimicrobials, 59.6% were given intravenous (range hospitals: 42.2 – 75.9).

![Figure 1: Prevalence of Antimicrobial Therapy per participating hospital (Mean with 95% CI, in order of prevalence)](image-url)
The first antimicrobial agent was considered appropriate in 1,690 (72.6%) patients. In 149 (6.4%) patients the first antimicrobial agent was considered inappropriate. In 223 (9.6%) patients it was appropriate to give AMT but the choice of the agent was not according to the guidelines. In 265 (11.4%) patients no decision was made due to insufficient information. The second antimicrobial agent was considered appropriate in 384 patients (78.2% of the 491 patients treated with more than 1 antimicrobial agent), inappropriate in 26 (5.3%) and appropriate but an incorrect choice in 39 (7.9%) patients. In 42 (8.6%) patients no choice was made due to insufficient information. The third antimicrobial was considered appropriate in 50 (86.2% of the 58 patients treated with 3 antimicrobial agents) patients, inappropriate in 2 (3.4%) and appropriate but incorrect choice in 2 (3.4%) patients. Four (6.9%) patients were not judged due to insufficient information.

Judgment of the appropriateness of AMT per patient showed that 372 patients (16% of the patients on AMT; 4.7% of the total population) were treated inappropriately. Figure 2 shows the variations in the proportion of AMT that is considered inappropriate in the different hospitals (range 5.0 - 32.4).

For 265 patients (11.4%) on AMT it was not possible to judge the appropriateness because of insufficient information. Figure 2 shows the variations in the proportion of cases with could not be judged in the participating centres (range 1.3 – 36.2). In hospital 1 the proportion of not-judged patients (27%) is more than twice as high as the proportion considered inappropriate (12%). In contrast, in hospital 7 only 3% of the patients could not be judged due to lack of information.

Of the patients without AMT (total 5,526), 945 patients were not judged for appropriateness of use. From the 4,581 patients without AMT who were judged on appropriateness, 4,497 (98.2%)
were considered appropriate. Twenty-two (0.5% of the judged patients without AMT) patients did not receive AMT, although this was indicated. For 62 (1.4%) patients without AMT it was not possible to judge the appropriateness because of insufficient information.

In table 2 the distribution of the use of various antimicrobials is shown. Co-amoxiclavulanic acid is the most commonly used antimicrobial agent, second are the fluoroquinolones and third are the 3&4th generation cephalosporins.

### Determinants of inappropriate use of antimicrobials

In the univariate analysis, the use of fluoroquinolones was statistically significant associated with more frequent inappropriate use of AMT (RR=1.4, Figure 3). The use of β-lactamase sensitive penicillin was significantly associated with more frequent appropriate use of AMT (RR=0.3, Figure 3).

Considering the use of AMT in the different medical specialties, Urology (p=0.002) proved to be statistically significant associated with more frequent inappropriate use (Figure 4). None of the specialties was statistically significant associated with a more frequent appropriate use.

Other factors that were statistically significantly associated with more appropriate use of AMT were: presence of a central venous catheter, presence of a peripheral vascular catheter, presence of an arterial catheter, being on the ICU and the presence of a hospital associated infection (Figure 5). Presence of a urinary tract catheter or an epidural catheter had no

<table>
<thead>
<tr>
<th>Table 2: Distribution of the use of antimicrobial agents per group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First Antibiotic</strong></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td><strong>N</strong></td>
</tr>
<tr>
<td>β-lactamase sensitive penicillins</td>
</tr>
<tr>
<td>β-lactamase resistant penicillins</td>
</tr>
<tr>
<td>carbapenem</td>
</tr>
<tr>
<td>co-amoxiclavulanic acid</td>
</tr>
<tr>
<td>1st generation cephalosporins</td>
</tr>
<tr>
<td>2nd generation cephalosporins</td>
</tr>
<tr>
<td>3rd&amp;4th generation cephalosporins</td>
</tr>
<tr>
<td>penicillin with extended spectrum</td>
</tr>
<tr>
<td>co-piperacillin/tazobactam</td>
</tr>
<tr>
<td>trimethoprim-sulfamethoxazole</td>
</tr>
<tr>
<td>quinolones</td>
</tr>
<tr>
<td>glycopeptides</td>
</tr>
<tr>
<td>imidazole derivates</td>
</tr>
<tr>
<td>lincosamides &amp; macrolides</td>
</tr>
<tr>
<td>other antimicrobials</td>
</tr>
<tr>
<td>aminoglycosides</td>
</tr>
<tr>
<td>tetracyclines</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
Chapter 2.2

B-lactamase sensitive penicillins
B-lactamase resistant penicillins
1st gen. cephalosporins
carbapenems
cO-amoxy-clavulanic acid
2nd gen. cephalosporins
3&4rd gen. cephalosporins
aminoglycosides
lincosamides & macrolides
sulfonamides & trimethoprim
quinolones

**Figure 3:** Relative Risk for Inappropriate use of Antimicrobial Therapy in groups of antimicrobial agents (co-amoxycillin-clavulanic acid as reference)

Cardio Thoracic Surgery
General Surgery
Lung diseases
Orthopaedics
Internal medicine
Neurology
Gynaecology
Urology

**Figure 4:** Relative Risk for Inappropriate use of Antimicrobial Therapy in medical specialties (internal medicine as reference)

**Figure 5:** Odds Ratio for Inappropriate use of Antimicrobial Therapy, determinants; hospital associated infections, medical devices, isolation precautions and the presence of ESBL-producing Gram Negative Rods
association with the appropriateness of AMT (Figure 5). The presence of a suprapubic catheter was statistically significantly associated with more inappropriate use of AMT (RR 1.9, Figure 5).

In multivariate analyses, taking the effects of all aforementioned variables into account, we found that the hospitals itself were important determinants associated with appropriate or inappropriate use (Table 3).

Furthermore, older age (p=0.024), ICU ward (p=0.002), central venous catheter (p=0.12), peripheral vascular catheter (p=0.005), hospital associated infection (0.049) and β-lactam sensitive penicillin use (p=0.017) were significantly associated with appropriate use in multivariate analyses. The presence of a suprapublic catheter (p=0.017) or the use of fluoroquinolones (<0.001) were associated with inappropriate use of AMT. No co-linearity was found between the variables in the multivariate model.

Table 3: Relative Risk for inappropriate use of Antimicrobial Therapy (multivariate analyses)

<table>
<thead>
<tr>
<th>Participating Hospital</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.07 (0.16 - 0.29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.11 (0.05 - 0.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.21 (0.07 - 0.70)</td>
<td>0.011</td>
</tr>
<tr>
<td>4</td>
<td>0.48 (0.87 - 0.81)</td>
<td>0.006</td>
</tr>
<tr>
<td>5</td>
<td>0.68 (0.27 - 1.71)</td>
<td>N.S.</td>
</tr>
<tr>
<td>6</td>
<td>0.93 (0.46 - 1.84)</td>
<td>N.S.</td>
</tr>
<tr>
<td>7</td>
<td>0.95 (0.46 - 1.94)</td>
<td>N.S.</td>
</tr>
<tr>
<td>8</td>
<td>0.95 (0.59 - 1.53)</td>
<td>N.S.</td>
</tr>
<tr>
<td>9</td>
<td>0.99 (0.54 - 1.82)</td>
<td>N.S.</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.05 (0.56 - 1.96)</td>
<td>N.S.</td>
</tr>
<tr>
<td>12</td>
<td>1.06 (0.62 - 1.79)</td>
<td>N.S.</td>
</tr>
<tr>
<td>13</td>
<td>1.11 (0.67 - 1.82)</td>
<td>N.S.</td>
</tr>
<tr>
<td>14</td>
<td>1.13 (0.51 - 2.54)</td>
<td>N.S.</td>
</tr>
<tr>
<td>15</td>
<td>1.27 (0.70 - 2.29)</td>
<td>N.S.</td>
</tr>
<tr>
<td>16</td>
<td>1.34 (0.71 - 2.53)</td>
<td>N.S.</td>
</tr>
<tr>
<td>17</td>
<td>1.89 (0.93 - 3.86)</td>
<td>N.S.</td>
</tr>
<tr>
<td>18</td>
<td>2.01 (1.22 - 3.31)</td>
<td>0.006</td>
</tr>
<tr>
<td>19</td>
<td>2.64 (1.20 - 5.80)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medical Device</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU ward</td>
<td>0.29 (0.14 - 0.64)</td>
<td>0.002</td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>0.54 (0.33 - 0.87)</td>
<td>0.012</td>
</tr>
<tr>
<td>Peripheral vascular catheter</td>
<td>0.70 (0.55 - 0.90)</td>
<td>0.005</td>
</tr>
<tr>
<td>Suprapubic catheter</td>
<td>3.52 (1.26 - 9.85)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

| Older Age     | 1.01 per year | 0.024   |
| Hospital associated infection | 0.68 (0.47 - 1.00) | 0.049   |

<table>
<thead>
<tr>
<th>Antimicrobial Use</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactamase sensitive penicillin</td>
<td>0.28 (0.10 - 0.80)</td>
<td>0.017</td>
</tr>
<tr>
<td>Quinolones</td>
<td>1.82 (1.35 - 2.45)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

N.S.: Not Significant
DISCUSSION

We researched the prevalence of AMT, and according to our study the mean prevalence of AMT was 29.6% (range 20.8 – 39.5%) in the Netherlands. The most recent study to compare this outcome with is the study from the ESAC II Hospital Care Study group, and they found a similar prevalence of 30% (range 19 –59%) in 20 European hospitals in 2006 [2]. In Sweden, a country comparable to the Netherlands considering the antimicrobial use, the STRAMA program reported an almost identical prevalence (30% in 2003-2004, unpublished data). Other publications show a wide variation in the prevalence of AMT [9-11].

Although our overall prevalence is comparable to other large surveys, there were large variations between the participating hospitals. This range of use can be explained by differences in patient populations and by differences in prescription policies.

Of all patients on AMT in this study, 371 (16%; range 1.9-27.7%), were considered inappropriate treated. The latter comprises 4.7% of the total group of patients, which may seem relatively unimportant. However, this means that annually approximately 10,000 days of unjustified AMT are given in a hospital with 200,000 patient days a year. This is associated with higher costs, more side effects and more antimicrobial resistance [12,13].

Figure 2 shows the differences per hospital in inappropriate judged treatment and the group of patients (265 in total) that could not be judged due to insufficient information according to the microbiologist. Apparently classification in appropriate or inappropriate is often not easy. However, the difference between the hospitals is remarkable. The hospitals with the lowest proportion of cases that could not be judged were the hospitals with previous experience with this kind of surveys. Possibly these kinds of judgments requires a more extensive training to deliver complete and reliable results.

Statistical analyses showed that the participating hospital is a risk factor it self, and this had a great influence in the analyses of determinants associated with inappropriate or appropriate use. Nevertheless, the use of fluoroquinolones proved to be a statistically significant risk factor for inappropriate use of AMT. And the use of β-lactamase sensitive penicillin was statistically significant associated with appropriate use.

Fluoroquinolones were the second most used antimicrobials in the prevalence survey. The ESAC reports that the fluoroquinolones showed the most dynamic increase in use, with growth rates of ≥15% in almost half of all countries [14]. At the same time the resistance against the fluoroquinolones increased from 5% (2001) up to 14% (2008) in *Escherichia coli* and from 4% (2005) up to 8% (2008) in *Klebsiella pneumoniae* [15].

This implicates the importance to start targeted interventions to reduce the inappropriate use of fluoroquinolones. And data from prevalence surveys like this can be helpful in these.
Other determinants associated with a more appropriate use of AMT were variables associated with the complexity of the patients (e.g. admission on ICU ward, central venous catheter). In the Netherlands, in the complicated patients usually a microbiologists or infectious disease physician is consulted. And the most patients who were considered inappropriately treated were the patients in whom the microbiologist or ID physician was not involved; the less complicated patients. This means that the focus should be on this group of patients to reduce the bulk of inappropriate AMT use.

The patients who were not judged may affect the outcome of our study and is therefore a weakness of the study. To investigate the affect a sensitivity analysis was performed. The univariate and multivariate analysis were repeated, once with all not judged patient’s categories as appropriate use and once with all not judged patients as inappropriate use. This affected the conclusions about the appropriateness of use in the participating hospitals. Therefore we cannot draw conclusions about the differences between most of the participating hospitals. However, two hospitals were significantly associated with more appropriate use in all 3 analyses and one hospital was significantly associated with a more inappropriate use in all 3 analyses.

The other determinants, i.e. medical devices, hospital associated infection and antimicrobial use were not affected in the sensitivity analysis and therefore we consider them valid.

In this study we identified those patients who inadvertently did not receive AMT (22 patients, 0.3% of total study population). In the single centre study from Willemsen et al. a similar fraction of inappropriately not treated patients was found (25 patients, 0.6% of total study population) and further investigation showed that those patients were not adversely affected at discharge [3]. Therefore, we decided to abandon further research on those 22 patients.

The amount of intravenous use (59.6%) is a possible target for improvement. Intervention studies performed in the Netherlands showed that intravenous use can be reduced relatively easy by targeted interventions [16,17]. This often resulted in a shorter hospitalisation. The appropriateness of the route of administration was not judged in this study.

The conclusion of this study was that it is possible to collect prevalence data on AMT on a national level, and that the individual hospital data of the appropriateness of antimicrobial use can be very helpful to initiate targeted interventions to improve antimicrobial use [17]. However the group not judged patients has to be reduced to produce more reliable results. Therefore training of ICP and consultant-microbiologist has to be intensified as well as training of medical staff in recording information to achieve a clearer and unambiguous assessment of AMT.
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Highly Resistant Micro-organisms;
Determinants for Incidence of Resistance and Transmission
Correlation Between Antibiotic Use and Resistance in a Hospital: Temporary and Ward-Specific Observations

Ina Willemsen¹, Diana Bogaers-Hofman¹, Marjolein Winters², Jan Kluytmans¹,³

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ABSTRACT

Objectives
The objectives of this study were to determine the increase in antimicrobial resistance to frequently used antibiotics in the hospital setting over time and the correlation between the amount of use of an antibiotic in a specific medical specialty and the observed resistance to that antibiotic in that specialty.

Method
The total use of antibiotics and the use of ciprofloxacin (CIP), co-amoxiclavulanic acid (AMCL) and first and second-generation cephalosporins (CEF) in individual medical specialties were measured between 2001 and 2006 by means of prevalence surveys (two per year). The antimicrobial susceptibility patterns among E. coli isolated from hospitalised patients between 2003 and 2006 were obtained from the Laboratory Information System. Trends over time and correlation between use and resistance were calculated.

Results
6,639 patients were included in the prevalence surveys, of whom 3.0% (195) were treated with CIP, 9.7% (642) with AMCL, and 3.5% (232) with CEF. 4,790 E. coli isolates were obtained from hospitalised patients. Resistance to all antibiotics significantly increased over time, with the regression line showing that the strongest increase in resistance was for CIP (2.6% per year).

There were large variations in antimicrobial use between various medical specialties. A significant correlation was found between the ward-specific prevalence of use and the percentage of resistance for CIP ($R = 0.81, p < 0.001$) and AMCL ($R = 0.82, p = 0.003$).

Conclusion
At the level of individual medical specialties within one hospital, a higher prevalence of antimicrobial use among patients was associated with a significantly higher observed antimicrobial resistance. The use of CIP was associated with a stronger increase in resistance than the use of beta-lactams.
INTRODUCTION

The threat to human health posed by antimicrobial resistance is of growing concern [1, 2]. Many commensal and pathogenic micro-organisms have developed resistance to antimicrobial agents [3]. In general, the development of resistance to micro-organisms is associated with the amount of use of antimicrobial agents, as shown repeatedly in studies at national and regional levels [4, 5]. The use of antimicrobial agents in the entire hospital correlates with the observed level of resistance [6].

However, the relationship between the amount of use within individual medical specialties of a hospital and the observed level of resistance in this medical specialty has not been clearly established. Harbarth et al. [7] investigated this relationship but was unable to find a significant relation for most of the antibiotics studied. It is important to have a good understanding of the relationship between the amount of use of antibiotics on individual wards and the development of antimicrobial resistance as such information would support targeted intervention programs, such as ward-specific or antimicrobial agent-specific interventions.

The objectives of the study were to determine the increase in resistance over time for the most frequently used antimicrobial agents in our hospital and to determine if the amount of antimicrobial use correlates with the development of resistance in \textit{Escherichia coli} in individual medical specialties.

MATERIALS AND METHODS

Hospital Setting

The Amphia hospital is a 1,370-bed teaching hospital, in which all medical specialties are present. In 2005, there were 40,525 admissions and 265,665 patientdays.

Use of Antimicrobial Agents

To determine the use of antimicrobial agents per medical specialty, ten consecutive 1-day prevalence surveys were performed between 2001 and 2006 – two prevalence surveys per year, in the spring and autumn, respectively. The reliability of the prevalence surveys to quantify the use of antimicrobial agents was validated in an earlier study [8]. In this study, prevalence of use corresponds to the prescribed daily doses (PDD).

In the prevalence surveys, the use of antimicrobial therapy was measured in all patients who were present in the hospital at 6 a.m. on the day of the survey; patients in daycare, in psychiatric wards, or on hemodialysis were excluded. The three most frequently used antimicrobial agents that are active against Gram-negative rods were included in the analysis: ciprofloxacin (CIP), co-amoxiclavulanic acid (AMCL), and first and second-generation cephalosporins (CEF), such as cefazolin, cefamandole, and cefuroxime (CFRX).
The prevalence of antimicrobial use is expressed as the number of patients using a specific antimicrobial agent on the day of the survey divided by the total number of patients per medical specialty included in this survey.

**Antimicrobial Resistance**

The susceptibility patterns of *E. coli* isolated from hospitalised patients between 2003 and 2006 were obtained from the Laboratory Information System (LIS). *E. coli* was used as the target micro-organism because it is the most common Gram-negative micro-organism isolated in nosocomial infections in our hospital.

Susceptibility patterns before 2003 were not included in our study because prior to 2003 another laboratory method was used. Antimicrobial susceptibility testing was performed with an automated system (Vitek; Biomerieux, Marcy-l’Etoile, France).

Interpretation of the antimicrobial susceptibility test results was based on guidelines from the Clinical and Laboratory Standards Institute (CLSI [formerly known as the NCCLS], Wayne, PA) [9]. Repeat isolates cultured from a patient after recovery of the initial isolate were excluded from analysis, unless there was a major difference in the susceptibility pattern. A major difference was defined as > 2-point difference in susceptibility pattern within 7 days and > 3-point difference within the whole period (1 point indicates a difference between intermediate and susceptible, or intermediate and resistant).

The percentage of resistance to CIP, AMCL, and CFRX in *E. coli* isolates was calculated per medical specialty and over time. Analyses were performed twice; in the first analysis, intermediate susceptibility was considered as indicating susceptible, and in the second analysis, intermediate susceptibility was considered as indicating resistant. The trend of resistance to CIP, AMCL, and CFRX over time in the period July 2003 until July 2006 was calculated, including *E. coli* isolates from patients who were hospitalised on the day the culture was taken. We also calculated the correlation between the pooled use of CIP, AMCL, and CEF over the period 2001–2006 and the subsequent pooled resistance to CIP, AMCL, and CFRX in the period July 2003–July 2006 in individual medical specialties. The analyses were repeated with the pooled use of CIP, AMCL, and CFRX over the period July 2003–July 2006 and the resistance rates from the same period. Because CIP is the only fluoroquinolone used in our hospital, only resistance to and use of CIP were determined. *E. coli* infection was considered as hospital acquired if the isolates were obtained > 48 h after admission.

**Data Analyses and Statistics**

Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS ver. 12.0; SPSS, Chicago, IL). Trends over time were examined using linear regression analysis. The Pearson correlation coefficient was calculated, and statistical significance was accepted when the chance for coincidence was < 5%.
RESULTS

A total of 6,639 patients were included in the prevalence surveys between 2001 and 2006, of which 23% (1,528) were on antimicrobial therapy. Of these, 3.0% (195) were treated with CIP, 9.7% (642) with AMCL, and 3.5% (232) with CEF. Regression analyses showed no significant changes in the total use of these antibiotics (p=0.154) and in the use of CIP (p=0.861) over the years. However, the use of AMCL (p=0.009) and CEF (p=0.001) did increase significantly during that period. Analysis of the pooled prevalence of antimicrobial use between 2001 and 2006 revealed large variations between various medical specialties, as is shown in table 1. CIP use was the highest in urology (8.7%) but not used at all in gynaecology; AMCL use was the highest in pulmonary diseases (23.7%) and lowest in orthopaedic surgery (2.5%); CEF use was the highest in urology (8.2%) and lowest in cardiology and neurology (0.9%).

There were 4,790 E. coli isolates obtained from hospitalised patients between July 2003 and July 2006, which represents 36% of all Gram-negative micro-organisms isolated from patients admitted to the Amphia hospital during this period. The mean number of E. coli isolates determined per quarter year was 399 (range 382–420), and no significant change was observed over time (Table 2). There was a significant increase in resistance over time for all antimicrobial agents (CIP, p < 0.001; AMCL, p = 0.031; CFRX, p = 0.012). Table 2 shows the number of resistant isolates per quarter year. The increase in microbial resistance against CIP, AMCL, and CEF over time is shown in figure 1. The strongest increase was observed for CIP, which showed an average annual increase of 2.6%; in comparison, the mean annual increase in microbial resistance to AMCL and CFRX was 1.5% and 1.9%, respectively.

Table 1: Pooled prevalence of antimicrobial use between 2001 and 2006 and pooled resistance in E.coli between July 2003 and July 2006 in individual specialties

<table>
<thead>
<tr>
<th>Medical specialty</th>
<th>Prevalence use (%)</th>
<th>Resistance (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CIP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>AMCL&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urology</td>
<td>8.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Geriatrics</td>
<td>5.1</td>
<td>8.5</td>
</tr>
<tr>
<td>Pulmonary diseases</td>
<td>4.2</td>
<td>23.7</td>
</tr>
<tr>
<td>Orthopaedic surgery</td>
<td>3.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Internal medicine</td>
<td>5.1</td>
<td>13.2</td>
</tr>
<tr>
<td>Surgery</td>
<td>3.0</td>
<td>11.0</td>
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<tr>
<td>Cardiology</td>
<td>0.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Paediatrics</td>
<td>0.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Gynaecology</td>
<td>0.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Neurology</td>
<td>1.3</td>
<td>5.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>CIP: ciprofloxacin; <sup>b</sup>AMCL: co-amoxy-clavulanic acid; <sup>c</sup>CEF: first and second generation cefalosporins; <sup>d</sup>CFRX: cefuroxime
Table 2: Total number of *E. coli* and of resistant isolates per quarter between July 2003 and July 2006

<table>
<thead>
<tr>
<th>quarter</th>
<th>Total isolates (N)</th>
<th>CIP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AMCL&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CFRX&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>406</td>
<td>16</td>
<td>17</td>
<td>20</td>
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<tr>
<td>2</td>
<td>374</td>
<td>21</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>398</td>
<td>15</td>
<td>26</td>
<td>14</td>
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<td>4</td>
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<td>27</td>
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<td>5</td>
<td>371</td>
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</tr>
<tr>
<td>12</td>
<td>399</td>
<td>38</td>
<td>38</td>
<td>23</td>
</tr>
</tbody>
</table>

<sup>a</sup>CIP, ciprofloxacin; <sup>b</sup>AMCL, co-amoxicillin-clavulanic acid; <sup>c</sup>CFRX, cefuroxime

Table 2 shows the use of CIP, AMCL, and CEF between 2001 and 2006 and the percentage of resistant *E. coli* isolates between July 2003 and July 2006 in individual specialties. Antimicrobial resistance to CIP was highest in urology (19.8%), while resistance to AMCL and CFRX was highest in lung diseases (11.2%) and urology (8.9%), respectively.

There was a significant relation between the prevalence of use between 2001 and 2006 and the observed resistance to CIP (R = 0.81, p=0.005) and AMCL (R = 0.82, p=0.003) between July 2003 and July 2006 in individual specialties, as shown in figure 2. A relative low amount of use was associated with a much higher observed resistance to CIP compared to AMCL. There was no significant relation between CEF use and CFRX resistance (R = 0.61, p=0.06). The outcome of these associations was similar when only the antimicrobial use data from the period July 2003 until July 2006 were included (CIP, R = 0.75, p= 0.013; AMCL, R = 0.80/ p=0.006).

The results of an analysis using only the hospital-acquired isolates (isolated at least 48 h after admission) obtained the same results as the other analyses (CIP, R = 0.78, p=0.01; AMCL, R = 0.82, p=0.004; CEF/CFRX, R = 0.23, p=0.56).
As a last step, the analyses were repeated including intermediate susceptible isolates to the resistant group. This did not change the overall results (results not shown).

**DISCUSSION**

A significant increase in antimicrobial resistance to CIP, AMCL, and CFRX over a relatively short period of time was observed, with the increase of resistance to CIP being stronger than that for AMCL and CFRX. This is a notable result considering that (1) the use of CIP was more or less stable over our study period, whereas the use of AMCL and CEF increased significantly and (2) the density of use of AMCL in the Amphia hospital was more than threefold higher than that of CIP.

Based on our results, it would appear that fluoroquinolones are more prone to lead to resistance in micro-organisms than beta-lactams. A high level of CIP resistance in relation to its usage has been reported earlier [10–12]. The 2006 European Antimicrobial Resistance Surveillance System report found that only Norway among European countries was able to keep their resistance level against fluoroquinolones in *E. coli* below 5%, with 11 countries reporting fluoroquinoloneresistant *E. coli* rates above 25% [12]. The increase observed in our study is stronger than that observed in other countries in previous studies. In a study carried out in the USA between 1997 and 2000, Lautenbach et al. found an increase in fluoroquinolone resistance in *E. coli* isolated from hospitalised patients of approximately 1.8% per year [13]. Another study from the USA reported an increase of resistance to CIP among aerobic Gram-negative bacilli in the...
period 1994–2000 of approximately 1.7% per year [14]. There is also a trend in the Netherlands towards increasing fluoroquinolone resistance in *E. coli* (from 3% in 2000 to 6% in 2006) [12]. This rapid increase likely represents an evolutionary phase in which resistance is increasing from a low level of resistance towards an equilibrium at a much higher level.

We also observed a significant correlation between the amount of use of CIP and AMCL, respectively, and antimicrobial resistance rate to these two antibiotics in individual medical specialties (CIP, \( R=0.81, p=0.005 \); AMCL, \( R=0.82, p=0.003 \)). For CFRX, no significant correlation between the amount of use (CEF) and the observed resistance to CFRX was found. A possible explanation for this is that first- and second-generation cephalosporins are frequently used for antibiotic prophylaxis. The dosing regimes for prophylaxis (short duration and relatively high dosage) are different from therapeutic applications.

The main conclusion that we draw from this study is that the use of fluoroquinolones is associated with more rapid development of resistance than the use of beta-lactam antibiotics.

Is this conclusion justified considering the methods that were used in this study? First, to determine the correlation between use and resistance, we made the assumption that antimicrobial usage precedes the development of resistance. From a theoretic point of view, this assumption is logical and is supported by several publications that show a delay between the use of antibiotics and the development of resistance [15]. For resistance in *Streptococcus pneumoniae*, Van Eldere et al. [16] found a lag period of 1 year between antibiotic use and observed resistance. In our study, a time period of 2 years was used. The analyses were then repeated, excluding the data on usage from 2001 to 2002. The findings from both analyses were similar.

Second, for the analysis of the individual specialties, we pooled the *E. coli* isolates and the use of antimicrobial agents. It would be interesting to break down the numbers per year, but this did not result in a useful analysis due to the fact that the numbers per subgroup were too small. During the study period no changes were made in the antimicrobial stewardship policies in our hospital and interventions to improve the use of antimicrobials started only after the study was completed.

Third, the influence of antimicrobial use outside the hospital on antimicrobial resistance was not taken into account. Partial adjustment was achieved by including only the hospital-acquired isolates, which resulted in similar conclusions. However, this method will not entirely control for the effect of antimicrobial use in the community [17, 18].

Finally, because all data were acquired from one hospital, the emergence of a resistant clone could influence the results of the study. In another study that we recently performed, all resistant *E. coli* strains from hospitalised patients were routinely typed using amplified fragment length polymorphism. A highly heterogeneous pattern was found, and there was not a single clone that had spread in our hospital. Upon weighing these four different aspects, we consider our conclusion justified.
The question that arises next is whether this process of development of resistance is reversible. Oteo et al. [19] investigated the evolution of community use of fluoroquinolones and trimethoprim-sulfamethoxazole (SXT) in comparison with the development of resistance to CIP and SXT in invasive community-acquired *E. coli* infections. Both the use and resistance of fluoroquinolones increased during the study period (2001–2003). However, although SXT use was strongly reduced from 1985 to 2003, SXT resistance showed only a slight decrease between 2001 and 2003. The authors speculated that this was most likely because SXT resistance in *E. coli* is often transferred by resistance genes that share other resistance mechanisms. Therefore, the decreased use of SXT may be compensated for by the use of other agents that also select for SXT resistance [20]. These mechanisms make it very difficult to reverse the process of antimicrobial resistance once it has been established. At the present time, it is unclear whether resistance, once it has been established, can be reversed. Therefore, it is of utmost importance to prevent the development of resistance in an early stage.

The correlation between hospital-wide antimicrobial use and fluoroquinolone resistance among inpatients has been described previously [13, 21, 22]. However, our study shows that even at the level of individual medical specialties within one hospital, a higher prevalence of CIP use is associated with the observed rate of resistance. Based on our findings, we feel that it is justified to develop targeted interventions on specific wards.

Furthermore, since the use of CIP has been shown to be associated with a stronger increase in antimicrobial resistance over time than the use of beta-lactams, a more restrictive use of fluoroquinolones should be a priority in antimicrobial prescription improvement programs.

ACKNOWLEDGMENTS

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REFERENCES


Highly Resistant Micro-organisms in a Teaching Hospital: The Role of Horizontal Spread in an Endemic Setting

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ABSTRACT

Objective
The objectives of this study were to determine the incidence density of Highly Resistant Microorganisms (HRMO) and the relative contribution of horizontal spread in a setting of endemicity.

Methods
A prospective surveillance was performed among hospitalised patients during an 18 months period. Enterobacteriaceae, Non-fermentative Gram negative rods, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecium* - all considered highly resistant, according to the Dutch guidelines - were included. Epidemiological linkage and nosocomial transmission were determined on the basis of molecular typing and hospital admission data.

Results
From 119 patient, we recovered a total of 170 unique HRMO isolates, as follows: *Escherichia coli*, 96 isolates; *Klebsiella* spp., 11 isolates; *Enterobacter* spp. 8 isolates; *Proteus* spp, 9 isolates; *Citrobacter* spp., 5 isolates; *Pseudomonas* spp., 5 isolates; *Acinetobacter* spp., 3 isolates; *Morganella* spp., 2 isolates; *Salmonella* spp., 1 isolate; *Serratia* spp., 1 isolate; *S. pneumoniae*, 20 isolates and *S. aureus*, 9 isolates. No vancomycin resistant *E. faecium* was found. The incidence density was 4.3 HRMO isolates per 10,000 patient days. The majority of HRMO isolates were unique and nosocomial transmission was observed 4 times for highly resistant Gram negative rods (case reproduction rate, 0.05) and 4 times for penicillin-non susceptible *S. pneumoniae* (case reproduction rate 0.29). A stay on the intensive care unit was the main determinant for recovery of an HRMO.

Conclusion
Nosocomial transmission of HRMO was observed 8 times during the 18 months period. The intensive care unit was identified as the main reservoir of horizontal spread of HRMO. This study shows that nosocomial transmission of HRMO is largely preventable using transmission based precautions.
INTRODUCTION

The spread of antimicrobial-resistant pathogens has become a major global problem, especially in hospitals. This is mainly caused by selection of drug-resistant strains resulting from the use of antimicrobial agents, and by transmission of drug-resistant strains between patients. To control the spread of drug-resistant strains, both processes should be taken into account [1-5]. The relative importance of these 2 mechanisms varies between hospitals and depends on the microbial species involved. To optimise control measures, it is important to know the relative contribution of both antimicrobial therapy and horizontal spread. Until now, most studies have focused on the role of antimicrobial therapy, whereas the role of clonal spread of highly resistant micro-organisms (HRMO) has been mainly studied in outbreak situations or in high-risk departments such as intensive care units (ICUs).

In 2005, definitions for HRMO were published by the Dutch Working Party on Infection Control [6,7]. The designation HRMO depends on the bacterial species and the antibacterial agent(s) which it has acquired resistance. Three main groups of HRMO are distinguished: highly resistant Enterobacteriaceae (including extended spectrum Beta-Lactamase (ESBL)-producing strains); highly resistant gram-negative nonfermentative bacteria; and highly resistant gram-positive bacteria. A summary of the definitions is provided in Table 1. Recommendations for use of isolation precautions, provided in the guideline, were implemented in our teaching hospital on January 1, 2005 [6].

The aim of this study was to determine both the incidence of HRMO and the relative contribution of clonal spread in a hospital, after the implementation of the guideline recommendations.

METHODS

The Amphia hospital is a 1,370-bed teaching hospital with 3 different locations. All medical specialities are available. In 2005, there were 40,525 admissions and 265,665 patient days. The average length of stay was 6.5 days.

A prospective surveillance was performed among hospitalised patients for 18 months, starting on January 1, 2005. Isolation precautions, as described in the guideline to prevent nosocomial transmission of HRMO, were used [6,7]. The Dutch “search and destroy” policy for Methicillin Resistant Staphylococcus aureus (MRSA), which includes active screening of high-risk patients, was also implemented [8,9].
Chapter 3.2

58

Collection of Infecting and Colonizing HRMO isolates:

The collection consisted of HRMO isolates recovered from specimens taken from hospitalised patients throughout the entire hospital. Most specimens were obtained on clinical indication of infection. In addition, on the ICU and the hemato-oncology ward, active screening was performed. These programs consisted of the collection of respiratory tract specimens on both wards and in addition rectal specimens on the hemato-oncology ward. Screening was performed on admission and twice per week thereafter. In case an HRMO was detected on a high risk ward (the ICU, the hemato-oncology ward, or, in case of *Streptococcus pneumoniae*, the pulmonary ward) in a patient who had not yet been appropriately isolated; all patients who had been in direct contact with the index patient were routinely screened for carriage of the species involved. Direct contact was defined as hospitalisation in the same room as the index patient. The results of contact tracing were also included in the study.

HRMO definition

*Enterobacteriaceae*, Non-fermentative Gram-negative bacilli, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecium* each fulfilled the criteria for HRMO, as defined in Table 1, and data on these pathogens were included in this study [6,7]. We defined the initial isolate as the first HRMO isolate recovered from a patient after admission to the hospital. Multiple isolates of a single species recovered from a single patient were only included if they showed major differences with respect to their susceptibility for co-amoxy-clavulanic acid, cephalosporins,

### Table 1: Definitions of Highly Resistant Micro-Organisms Used in the Study

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>FLOX</th>
<th>GLY</th>
<th>PEN</th>
<th>ESBL</th>
<th>CAR</th>
<th>QUI</th>
<th>AMG</th>
<th>CFT</th>
<th>PIP</th>
<th>TMP-SMZ</th>
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<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>A</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>A</td>
<td>A^a</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><em>Enterococcus faecium</em></td>
<td>B</td>
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<td></td>
<td></td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td></td>
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<td></td>
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<tr>
<td>other <em>Enterobacteriaceae</em></td>
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<td>A</td>
<td>B</td>
<td>A</td>
<td></td>
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<tr>
<td><em>Acinetobacter</em> spp.</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<td>C</td>
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<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>A</td>
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</table>

NOTE. These definitions are adapted from Kluytmans-VandenBergh et al.[6]

^a^ Includes intermediately resistant isolates (penicillin minimum inhibitory concentration, 0.1-1.0 mg/L)

FLOX: flucloxacillin; GLY: glycopeptides; PEN: penicillins; ESBL: Extended Spectrum Beta-Lactamases; CAR: carbapenems; QUI: fluoroquinolones; AMG: aminoglycosides; CFT: ceftazidim; PIP: piperacillin; TMP-SMZ: trimethoprim-sulfamethoxazole

**A**: resistance against an antibacterial agent from one of the indicated groups is sufficient to define the micro-organism as highly resistant

**B**: resistance against antibacterial agents from at least two of the indicated groups is required to define the micro-organism as highly resistant

**C**: resistance against antibacterial agents from at least three of the indicated groups is required to define the micro-organism as highly resistant

A H R M O  d e f i n i t i o n

*Enterobacteriaceae*, Non-fermentative Gram-negative bacilli, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecium* each fulfilled the criteria for HRMO, as defined in Table 1, and data on these pathogens were included in this study [6,7]. We defined the initial isolate as the first HRMO isolate recovered from a patient after admission to the hospital. Multiple isolates of a single species recovered from a single patient were only included if they showed major differences with respect to their susceptibility for co-amoxy-clavulanic acid, cephalosporins,
fluoroquinolones, carbapenems, aminoglycosides and TMP-SMZ. A major difference was defined as a test result of “susceptible” for 1 strain and “resistant” for the other strain.

**Patient data**

For each patient from whom we recovered an HRMO isolate, we recorded data on age, sex, medical speciality and dates of admission and of discharge. For each isolate, we recorded data on bacterial species, date of isolation, and antimicrobial susceptibility pattern.

All transfers of patients between medical wards were registered. Data on diagnostic or therapeutic procedures, such as surgery or radiology were not included in our study. Furthermore, it was registered whether the patient had been admitted to the ICU the 30 days prior to the first isolation of an HRMO.

The control group comprised patients from whom we recovered, during the study period, drug-susceptible isolates of the species included in the HRMO guideline. The identification of patient colonised or infected with susceptible isolates was performed in the same way as the identification of patients colonised or infected with an HRMO.

**Testing methods**

All susceptibility tests were performed according to the guidelines from the Clinical and Laboratory Standards Institute [10]. Susceptibility patterns of *S. aureus*, *E. faecium*, *Enterobacteriaceae* and non-fermentative gram-negative bacilli (GNB) were determined using the VITEK 2 system (Biomérieux). *S. aureus* strains that were resistant to beta-lactams *in vitro*, were subsequently tested for the presence of the *mecA* gene by polymerase chain reaction (PCR). The susceptibility of *S. pneumoniae* isolates to oxacillin was determined on the basis of microdilution and agar-diffusion. For all strains, suspected reduced susceptibility to penicillin was confirmed using the Etest (AB Biodisk).

For molecular typing, chromosomal DNA was isolated using the QIAMP minikit (QIAGEN). Amplified restriction fragment-length polymorphism (AFLP) was performed as described by Savelkoul et al. [3,11]. After restriction, ligation and amplification, the DNA fragments were separated by means of an ABI Prism 3100 Genetic Analyser (Applied Biosystems). The data were analysed with the Pearson correlation coefficient and clustered by unweighted pair-group matrix analyses by means of BioNumerics software, v3.0 (Applied-Maths).

**Definitions and epidemiological analysis**

The Incidence Density was calculated as the number of newly isolated micro-organisms divided by the total number of patient days in the study period. HRMO isolates recovered from a specimen obtained from a patient more than 72 hours after admission, or less than 72 hours after admission but shortly after a recent discharge (within 30 days) were classified as “hospital associated”.

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[99x633]Highly Resistant Micro-organisms in a Teaching Hospital: The Role of Horizontal Spread in an Endemic Setting

[424x633]59
HRMO isolates recovered from a patient who was on ICU, or who had been on an ICU during the 30 days prior to the recovery of the isolate, were classified as “ICU associated”. The genetic relatedness of isolates was determined on the basis of both visual and computerised interpretation of the AFLP patterns. Isolates with more than 90% correspondence were considered genetically related.

Epidemiological linkage was considered present if two patients had been in the same hospital ward within a maximum time window of 4 weeks. Nosocomial transmission was considered to have occurred if genotypically related strains were detected in epidemiologically linked patients. The case reproduction rate was calculated as the number of infections due to an HRMO and acquired by nosocomial transmission divided by the number of infections due to an HRMO of the same species and not acquired by nosocomial transmission.

**Data-analyses, quality control, and statistics**

The privacy of patients was protected by coding all data, according to the requirements of the privacy regulation in our hospital. The data were entered into a database and analysed using Excel and the Statistical Package for Social Sciences software, version 12.0 (SPSS). To assess differences in occurrence between the ICU-associated strains and the non-ICU-associated strains, relative risk and 95% confidence interval were calculated. The Fisher’s exact test (2-tailed) was used to determine statistical significance, which was considered to be present if the chance for coincidence was less than 5%.

**RESULTS**

**Hospital and Patient Data**

During the study period, there were 60,790 admissions, of which 6,310 (10%) were admissions to the ICU. The hospital had a total of 398,497 patient days (15,427 patient days [4%] were on the ICU). The median age of the patients was 65.5 year (range, 3-97 years), and 60% of the patients were male.

**HRMO**

A total of 170 unique HRMO isolated were recovered from 119 patients. Of these 170 isolates, 96 (56%) were *E. coli* isolates, 11 (6%) were *Klebsiella* spp, 8 (5%) were *Enterobacter* spp, 9 (5%) were *Proteus* spp, 5 (3%) were *Citrobacter* spp, 5 (3%) were *Pseudomonas* spp, 3 were *Acinetobacter* spp, 2 were *Morganella* spp, 1 was *Salmonella* spp, 1 was *Serratia* spp, 20 (12%) were *S. pneumoniae* isolates, and 9 (5%) were *S. aureus* isolates. No isolates of vancomycin-resistant *E. faecium* were recovered.

Most patients with an infection due to an HRMO were hospitalised on the surgery ward (52 [44%]), the ICU (43 [36%]), or to internal medicine ward (29 [24%]) at the time the initial
isolate was recovered. The median time between admission of a patient and recovery of the first HRMO isolate was 8 days (range, 0-65 days). Sixteen percent of patients died within 30 days after recovery of the HRMO isolate. The median length of stay of patient with an infection due to an HRMO was 17 (range, 2-93 days).

**Gram-Negative Bacilli**

*Incidence density*

One hundred forty-one highly resistant gram-negative bacilli (HR-GNB) isolates were recovered from 94 patients (Incidence density, 3.5 isolates per 10,000 patient days), whereas 6,178 non-highly resistant (non-HR)-GNB were isolated during the same period (Incidence density, 155 isolates per 10,000 patient days). Panresistant GNB isolates were not observed. Except for one *Acinetobacter* isolate, all HR-GNB isolates were susceptible to meropenem. One hundred eight (76.6%) of the HR-GNB isolates were recovered from 66 patients, and 3,924 (63.5%) of the non-HR-GNB isolates were recovered from 2204 patients, were hospital associated. The association of these isolates with a stay on the ICU is shown in Table 2. The relative risk for the recovery of an HR-GNB isolate was significantly higher for the patients who had an ICU stay.

Twenty HR-GNB isolates (14%) were recovered from rectal, throat and nasal swab specimens. All other HR-GNB isolates were recovered from clinical samples, as follows: blood (10 [7%]) of isolates; respiratory tract samples (23 [16%]); wounds samples (28 [20%]); urine (46 [33%]); and aseptically obtained aspirations samples (14 [10%]).

*Comparison of isolates recovered from a single patient*

For the 141 HR-GNB isolates, AFLP results were obtained for 109 isolates that were recovered from 85 patients (17 isolates were not available for testing, and AFLP analysis of 15 isolates led repeatedly to inconclusive results). Differences in susceptibility patterns indicated that 13 patients harboured more than one highly resistant *E. coli* strain.

Genotyping showed that, in 6 patients, the two *E. coli* strains were, in fact identical. Five patients carried two or more highly resistant *E. coli* strains that were unique, and DNA of the *E. coli* strains from 2 patients were not suitable for AFLP analysis (Figure 1). Isolates of all other species of HR-GNB that were recovered more than once from one patient were always genetically identical.
Comparison of isolates recovered from different patients

Six clusters of genetically related *E. coli* isolates recovered from different patients were found. Epidemiological linkage between patients was observed in 3 of the 6 clusters, and a total of 4 patients fulfilled the definition of nosocomial transmission (Figure 2). None of the cases of nosocomial transmission was found by contact tracing.
Two of the three clusters with epidemiological linkage involved patients who had stayed on the ICU for more than 30 days. The strain of the third cluster was found in 3 patients who had been on the same surgical ward.

Genetic relatedness was only observed in *E. coli* strains. Of a total of 85 patients with HR-GNB isolates that were available for typing, nosocomial transmission to 4 patients was detected, resulting in case reproduction rate of 0.05.

**Penicillin-Nonsusceptible *S. pneumoniae***

*Incidence density*

Twenty penicillin-nonsusceptible *S. pneumoniae* isolates were recovered from 19 patients (Incidence density, 0.5 isolates per 10,000 patient days). Two of the isolates were recovered from blood; all other isolates were recovered from respiratory tract specimens. During the same period 371 penicillin-susceptible *S pneumoniae* isolates were recovered (Incidence density, 9.3 isolates per 10,000 patient days).

Half of the penicillin-nonsusceptible *S. pneumoniae* and a quarter of the penicillin-susceptible *S pneumoniae* were hospital associated. The relative risk for penicillin-nonsusceptible *S. pneumoniae* was statistically higher for patient who stayed on the ICU than for those who stayed on non-ICU areas, as shown in Table 2.

**Comparison of isolates from different patients**

Nineteen penicillin-nonsusceptible *S. pneumoniae* isolates were available for AFLP typing, and two clusters were found. Cluster A involved 11 patients; cluster (B) involved 2 patients. Four isolates were unique.

Four patients in cluster A were epidemiologically linked, and nosocomial transmission was observed three times (Figure 3). Two of the three patients were identified by contact tracing. The isolates of cluster B were genetically related and epidemiologically linked, representing one case of nosocomial transmission. No active contact tracing was performed in this case.
AFLP typing of penicillin-nonsusceptible *S. pneumoniae* isolates from a total of 14 primary patients indicated that nosocomial transmission to 4 patients occurred resulting in a case reproduction rate of 0.29. This case reproduction rate was significantly higher than that for HR-GNB infection (0.05 (p=0.030)). All cases of nosocomial transmission occurred when patients were not yet under droplet precautions. Once droplet precautions were initiated not a single case of nosocomial transmission was observed.

**Figure 3:** Timeline showing the epidemiological linkage between 4 patients involved in cluster A of genetically related, penicillin-nonsusceptible *Streptococcus pneumoniae* isolates

CAR: cardiac surgery ward; CTS: cardiothoracic surgery ward; HRMO: highly resistant micro-organism; ICU: intensive care unit; INT: internal medicine ward; PUL: pulmonary disease ward; SUR: surgery ward

**MRSA and Vancomycin-Resistant Enterococci**

MRSA was isolated from nine patients; only 1 of these MRSA isolates was considered hospital associated. However the source remained unknown. Eight of the 9 isolates were available for molecular typing. This analysis revealed no genetic relatedness. No vancomycin-resistant *Enterococci* (VRE) were found during the study period.

**DISCUSSION**

The incidence density of isolation of HRMO in this study was 4.3 per 10,000 patient days. In 18 months there were 8 cases of nosocomial transmission (4 of HR-GNB and 4 of penicillin-nonsusceptible *S. pneumoniae*). There was no nosocomial spread of MRSA and VRE. The main source of HRMO in this setting is likely to be the endogenous flora of the patient already present on admission to the hospital [12].

However, these results might be biased because the study relied on information from samples obtained because of a clinical indication. Also, no information was available about patient risk factors, such as residence in a nursing homes or previous use of antimicrobial therapy.

The incidence density of HRMO infection in our study was difficult to compare with other findings or other studies because of the variation in the definition used. Most reports use resistance rates against single antimicrobial agents for selected species [13]. These data are relatively easy to obtain and easy to understand but also of limited value, because the problem faced in clinical
Highly Resistant Micro-organisms in a Teaching Hospital: The Role of Horizontal Spread in an Endemic Setting

Decision making is mainly multidrug resistance. In a study describing the endemic situation in a German hospital during a 3-year period, a similar incidence of isolation of multidrug-resistant gram-negative bacteria was found (4.3 / 10,000 patient days) [14]. In the German study, a multidrug-resistant GNB was defined as a Gram-negative aerobic rod determined to be susceptible to fewer than 2 groups of bactericidal antimicrobials, by means of in vitro tests. This definition is different from what we used. Our definitions are based upon both single-drug resistance and multidrug resistance, depending on the impact on clinical decision making [6]. Our definitions cover the entire spectrum of clinically relevant bacteria (excluding Mycobacterium species). For future studies and for benchmarking, it is important to use uniform definitions of an HRMO, therefore, an international standard is needed.

To determine the genetic relatedness of isolates within the different species of HRMO, AFLP was used. AFLP is a suitable typing method for this kind of analysis, because of its combination of high discriminatory power, capability to type various bacterial species, good reproducibility and production of clear banding patterns that can be objectively analysed using a computer [15].

AFLP analysis showed that the majority of HRMO isolates were unique. Comparison of AFLP results with epidemiological data revealed a limited spread of HR-GNB. Only 4 cases of nosocomial transmission were observed, resulting in a reproduction rate of 0.05, which means that, in 95% of the cases, transmission was not observed.

Our results show that nosocomial spread is under control and far from the breakpoint of ongoing transmission (ie, a case reproduction rate of greater than 1.0). We are not aware of comparable data from other investigators. In most studies, transmission of all organisms, drug-susceptible and drug-resistant, is investigated. For example Grundman et al. found that 14.5% of all nosocomial infections detected in patients on the ICU were associated with transmission between patients [16].

An unexpected finding from this study is that the control of transmission was significantly less effective for penicillin-nonsusceptible S. pneumoniae than for HR-GNB (p=0.030). First, it must be noted that half of the patients from whom penicillin-nonsusceptible S. pneumoniae were recovered (9 of 19 patients) had chronic obstructive pulmonary diseases; these patients were frequent users of antimicrobial agents and visited the hospital a mean of 3 times (range, 1-6 times) during the study period. These patients are known to be vulnerable to S. pneumoniae infections, in general, and even more vulnerable to penicillin-nonsusceptible S. pneumoniae infection, because of the frequent use of antimicrobial agents [17]. This may partly explain the relatively high case reproduction rate (0.29) that was found.

However, the timing of isolation precautions may be important as well. In our hospital, isolation is initiated once detection of a penicillin-nonsusceptible S. pneumoniae is reported by the laboratory. In all cases of nosocomial transmission, the index patients had not been treated
under droplet precautions, because laboratory results were not available yet [6,7]. Once these precautions were initiated, no further transmission was found.

On the basis of these results, droplet precautions seem to be effective in preventing nosocomial transmission of penicillin-nonsusceptible *S. pneumoniae*, but more rapid laboratory procedures to identify these organisms are needed to control nosocomial more effectively.

Most of the HRMO isolated (77% of the HR-GNB isolates, 64% of the penicillin-nonsusceptible *S. pneumoniae* isolates) were recovered from samples obtained more than 72 hours after admission and therefore considered hospital associated. This was not the case for MRSA. Most MRSA carriers (8 [89%] of 9 carriers) were placed in isolation on the day of admission, and, therefore the MRSA isolated recovered from these patients were considered not to be hospital associated. This finding is similar to the patterns of MRSA prevalence in other Dutch hospitals [18]. Nosocomial MRSA transmission did not occur, which confirms the effectiveness of the “search and destroy” policy in controlling the spread of MRSA in the hospital [19,20].

Transmission of VRE could not be detected, because no VRE isolates were recovered during the study period. VRE infection has been reported less frequently in Europe than in the United States, but carriage of VRE has been found in approximately 5% of healthy individuals in Europe [21, 22]. These strains are apparently not causing clinical problems. In this study, no active surveillance for gastrointestinal colonisation was performed, except in the hemato-oncology ward, which comprised only a small part of the study population.

There are some limitations to this study. First, it relied on routine clinical samples from patients, except for those on the ICU and the hemato-oncology ward, and, therefore, could have missed some cases of nosocomial transmission. This potential deficit was at least partially mitigated, because contact tracing was performed when a patient with a HRMO was identified on a high-risk ward. Contact tracing included all patients who had been in the same room, and who were still present in the hospital. Only 2 cases of nosocomial transmission of penicillin-nonsusceptible *S. pneumoniae* were found using contact tracing.

Thouverez et al. evaluated the efficiency of using screening cultures to control of ESBL-producing Enterobacteriaceae in ICU [23]. They observed a relative inefficiency of the screening test and they concluded that clinical cultures may be sufficient to control ESBL-producing Enterobacteriaceae in nonepidemic situation and to detect outbreaks. Another study in the United States observed a low rate of recovery of isolates with repeated screening of known carriers of ESBL-producing Enterobacteriaceae [24]. Nevertheless, an unknown number of the patients with HRMO might have been unnoticed.

The potential bias introduced by obtaining samples on clinical indication is demonstrated in Table 2. The relative risk of detection of HR-GNB in patients staying on the ICU, compared with patients staying on the non-ICU wards, is 16.1 (95% confidence interval, 9.9-26.3). However, on
the ICU, a more intensive screening policy is used, compared with other wards in the hospital, resulting in a higher likelihood to detecting an HRMO. To estimate the level of detection bias, we also calculated the ratio of the number of HR-GNB isolates divided by the total number of GNB isolates. According to this calculation the relative risk of isolation of an HR-GNB on the ICU, compared with the other wards in the hospital, is much lower, albeit still statistically significant.

For evaluation of epidemiological linkage, a period of maximum 4 weeks was chosen. Choosing a longer period would have made very little difference; no extra cases of nosocomial transmission were found with a period of maximum 8 weeks.

Finally, we present findings about horizontal transmission of bacteria only, excluding the possible role of mobile genetic elements, such as plasmids and integrons [17]. The role of mobile genetic elements was part of our study, as well, but is described in a separate publication; nosocomial spread of integrons among HR-GNB isolates was observed more frequently than the clonal spread of the entire bacterium [25]. The spread of mobile genetic elements is of increasing importance and requires additional laboratory methods to monitor it [12].

In conclusion; this study shows that nosocomial transmission of HRMO can be controlled effectively by means of transmission-based precautions, although it is uncertain how many more instances of nosocomial transmission would have occurred with less stringent transmission-based precautions. Further improvement of the control measures can be achieved by more rapid laboratory detection of resistant isolates. The ICU was identified as the main place of acquisition of HRMO and remains the primary target for control measures.
REFERENCES


Integron Class 1 Reservoir among Highly Resistant Gram-Negative Microorganisms Recovered at a Dutch Teaching Hospital

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ABSTRACT

Integrons play an important role in the dissemination of resistance genes among bacteria. Nearly 70% of highly resistant gram-negative bacteria isolated at a tertiary care hospital harboured an integron.

Epidemiologic analysis suggests that horizontal gene transfer is an important mechanism of resistance spread and has a greater contribution than cross-transmission to levels of resistance in settings where highly resistant gram-negative bacteria are endemic.
INTRODUCTION

The worldwide emergence of highly resistant micro-organisms is a matter of continuous concern. The Netherlands has adopted national guidelines to control the emergence and spread of antimicrobial resistance [1,2]. Traditionally, infection control focuses on the prevention of clonal dissemination of highly resistant micro-organisms.

Nowadays, we know that multidrug resistance is often associated with the spread of transmissible plasmids and integrons, the latter of which have been the subjects of more-recent investigation. Integrons encode a RecA-independent, site-specific integration system with the ability to capture mobile gene cassettes, notably those that contain genes encoding antimicrobial resistance. Bacteria carrying integrons are widespread in the community [3].

Our objective was to determine the extent to which integrons contribute to the spread of multidrug resistance in a clinical setting where highly resistance micro-organisms are endemic.

MATERIALS AND METHODS

Bacteria
During an 18-month period in 2005 and 2006, there were 136 highly resistant micro-organisms isolated from routine diagnostic specimens. Highly resistant micro-organisms were defined according to the criteria described by Kluytmans-Vandenbergh et al. [1] Genotypically identical strains isolated from a single patient were included in the analysis if they differed greatly from each other in their susceptibility to co-amoxy-clavulanic acid, cephalosporins, fluoroquinolones, carbapenems, aminoglycosides, and trimethoprim-sulfamethoxazole.

Integrons
Integrons were detected by amplification of the integrase genes of class 1 integron (intI1) and class 2 integron (intI2), using the primers Int1F/Int1R and Int2F/Int2R, respectively, as described elsewhere [4]. Subsequent amplification of the gene cassettes in strains that contained intI1 was performed with the primers 5’CS and 3’CS, followed by restriction analysis [4]. Conjugation experiments were performed as we described elsewhere [4].

RESULTS

Highly resistant micro-organisms were recovered from 109 patients. Gram-negative bacteria were recovered from 85 patients, and gram-positive bacteria were recovered from 25 patients. One patient was infected with gram-negative and gram-positive bacteria. Differences in drug-susceptibility profiles among strains recovered from individual patients yielded 136 highly
resistant strains for analysis. There were 109 strains of gram-negative bacteria and 27 strains of gram-positive bacteria. All 136 strains were further investigated using amplified fragment–length polymorphism (AFLP) analysis to determine the presence of class 1 and 2 integrons [5].

**Characteristics of class 1 integrons and intI1**

Of the patients infected with a gram-negative bacterium, 83 were infected with highly resistant Enterobacteriaceae strains, of which 76 (72%) showed reduced susceptibility to cephalosporins (defined as resistance to at least 1 antimicrobial agent from the cephalosporin group). Fifty-five percent of those patients (42 of 76) harboured a class 1 integron structure (Table 1).

<p>| Table 1: Characteristics of Gram-Negative Bacteria Recovered from Patients in a Dutch Teaching Hospital |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th><strong>Species</strong></th>
<th><strong>No. of strains recovered</strong></th>
<th><strong>No. of intI1-positive strains</strong></th>
<th><strong>Gene cassette(s) detected</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>2</td>
<td>2</td>
<td>...</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>3</td>
<td>3</td>
<td>IJ, TB, K</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>2</td>
<td>2</td>
<td>BL, N</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>3</td>
<td>3</td>
<td>IJ, TB, O</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>2</td>
<td>2</td>
<td>A, UN</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>5</td>
<td>5</td>
<td>C</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>4</td>
<td>4</td>
<td>N, P</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>3</td>
<td>4</td>
<td>H, O</td>
</tr>
<tr>
<td>Serratia plymuthica</td>
<td>1</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>2</td>
<td>2</td>
<td>IJ</td>
</tr>
<tr>
<td>Overall</td>
<td>91</td>
<td>109</td>
<td>76</td>
</tr>
</tbody>
</table>

**NOTE.** intI1, integrase contained in class I integrons.

*Uniqueness was determined on the basis of amplified fragment–length polymorphism analysis*

Seventy-six gram-negative strains were isolated from the 42 patients who were infected with a highly resistant, class 1 integron– harbouring Enterobacteriaceae with reduced susceptibility to cephalosporins, accounting for 69.7% of our gram-negative bacterial collection (76 of 109 strains).

Characterisation of the gene cassettes of all intI1-positive strains by restriction fragment–length polymorphism–polymerase chain reaction and sequence analysis showed 22 different gene cassettes, of which 10 were found only once (Table 2).

Two cassettes, designated A and B, were most prevalent. Gene cassettes A and B contained genes conferring trimethoprim and streptomycin–spectinomycin resistance, similar to all other gene cassettes types except 2 (types N and T). In addition, genes conferring resistance to chloramphenicol, blactams, and aminoglycosides (ie, kanamycin, gentamicin, amikacin, and erythromycin) were identified.
Results of the in vitro conjugation experiment showed that the multidrug resistance phenotype could be spread via plasmids harbouring class 1 integrons. To investigate whether this mechanism also played a role in vivo, we determined the epidemiologic linkage of integron transmission. Genotypically identical strains were omitted from this analysis because secondary transmission of these strains involves nosocomial transmission of the bacterium rather than integron transmission.

We showed that 7 of 59 patients were infected via horizontal transmission, yielding an intergron-transmission index of 12%. Six of 13 strains harbouring gene cassette B were involved in horizontal gene transfer, showing that this plasmid is highly mobile (5 cases of horizontal gene transmission).

### Table 2: Characteristics of 22 Gene Cassettes Found in Highly Drug-Resistant Gram-Negative Bacteria Containing Class 1 Integrons

<table>
<thead>
<tr>
<th>Gene cassette</th>
<th>Resistance phenotype</th>
<th>No. of strains with gene cassette</th>
<th>No. of patients (n=59)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>TMP, Stm-Spm</td>
<td>16</td>
<td>24</td>
<td>20 Escherichia coli, Morganella morganii</td>
</tr>
<tr>
<td>B</td>
<td>Stm-Spm, TMP</td>
<td>12</td>
<td>15</td>
<td>11 Enterobacter aerogenes, Enterobacter cloacae, Citrobacter freundii</td>
</tr>
<tr>
<td>C</td>
<td>Stm-Spm</td>
<td>2</td>
<td>2</td>
<td>2 E. coli, Proteus mirabilis</td>
</tr>
<tr>
<td>D</td>
<td>ND</td>
<td>3</td>
<td>3</td>
<td>3 E. coli</td>
</tr>
<tr>
<td>E</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 E. coli</td>
</tr>
<tr>
<td>F</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 E. coli</td>
</tr>
<tr>
<td>G</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 E. coli</td>
</tr>
<tr>
<td>H</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 Klebsiella pneumoniae</td>
</tr>
<tr>
<td>I</td>
<td>Gm-Km-Tm</td>
<td>3</td>
<td>4</td>
<td>2 C. freundii, E. cloacae, Proteus vulgaris</td>
</tr>
<tr>
<td>J</td>
<td>TMP, Em</td>
<td>3</td>
<td>4</td>
<td>2 C. freundii, E. cloacae, P. vulgaris</td>
</tr>
<tr>
<td>K</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 K. pneumoniae</td>
</tr>
<tr>
<td>L</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 E. aerogenes</td>
</tr>
<tr>
<td>N</td>
<td>Gm-Km-Tm, Chl</td>
<td>3</td>
<td>3</td>
<td>2 E. aerogenes, Klebsiella oxytoca, M. morganii</td>
</tr>
<tr>
<td>O</td>
<td>Stm-Spm, TMP</td>
<td>3</td>
<td>3</td>
<td>3 E. coli, E. cloacae, K. pneumoniae</td>
</tr>
<tr>
<td>P</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 K. oxytoca</td>
</tr>
<tr>
<td>S</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 E. coli</td>
</tr>
<tr>
<td>T</td>
<td>Amik, Stm-Spm</td>
<td>2</td>
<td>2</td>
<td>1 E. cloacae, C. freundii</td>
</tr>
<tr>
<td>U</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 M. morganii</td>
</tr>
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<td>W</td>
<td>ND</td>
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<td>1 E. coli</td>
</tr>
<tr>
<td>Y</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>1 E. coli</td>
</tr>
<tr>
<td>Z</td>
<td>ND</td>
<td>3</td>
<td>3</td>
<td>3 E. coli</td>
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</table>

NOTE. Amik: amikacin; Chl: chloramphenicol; Em: erythromycin; Gm: gentamicin; Imp: imipenem; ND: no data; Km: kanamycin; Spm: spectinomycin; Stm: streptomycin; Tm: tobramycin; TMP: trimethoprim.

*a Uniqueness was determined on the basis of amplified fragment–length polymorphism analysis

*b Two patients were infected with highly drug-resistant gram-negative bacteria. Therefore, the total number of patients is 59 instead of 61.
This observation contrasts with that for cassette A, in which integron transmission was detected for only 2 of 23 strains (1 case of horizontal gene transmission). The transmission index for cassette B was 46% and was significantly greater than the transmission index of 9% observed for cassette A (relative risk [RR], 5.3 [95% confidence interval {CI}, 1.4–21.4]).

In addition, we found a linkage for gene cassettes I and J, which were both present on the same plasmid. This plasmid was found first in 2 strains (*Citrobacter freundii* and *Proteus vulgaris*) isolated from a single patient and subsequently in 1 strain (*Enterobacter cloacae*) recovered from another patient in the same ward.

Of interest, integron transmission was only observed in patients admitted to intensive care units (ICUs). Epidemiologic linkage could not be observed for other gene cassettes.

During the study period, 60,790 patients were admitted to Amphia Hospital (Breda, the Netherlands), of whom 6,310 were admitted to the ICU. The overall cumulative incidence of integron transmission was therefore 1.2 cases per 10,000 patients (7 cases per 60,790 admissions). As expected, the cumulative incidence of integron transmission among patients in the ICU was much greater (at 11.1 cases per 10,000 patients) than the overall cumulative incidence.

**Prevalence of class 1 integrons in the ICU**

Thirty-six (85%) of 42 highly resistant gram-negative rods isolated from patients admitted to the ICU harbored a class 1 integron. Although more highly resistant gram-negative rods were isolated from non-ICU wards, the prevalence of class 1 integrons was lower (60% [40 of 67 highly resistant gram-negative strains recovered from non-ICUs]). Admission to the ICU was therefore associated with a greater risk of infection with a highly resistant gram-negative rod that harboured a class 1 integron (RR, 1.4 [95% CI, 1.1–1.8]).

**DISCUSSION**

Class 1 integrons were detected in nearly 70% of highly resistant gram-negative bacteria isolated in a teaching hospital over a period of 18 months. This suggests that there is a large reservoir of mobile elements that carry resistance genes in the microbial communities of hospitals.

It is known that integrons can be transferred readily between different species in vitro as well as in vivo, thereby facilitating the spread of multidrug resistance [6]. In our study, 3 patients were infected with 2 or more multidrug-resistant species that harboured identical integron structures. Moreover, 1 patient was infected with 2 genotypically different *Escherichia coli* strains containing gene cassette B. This strongly suggests that interspecies gene transfer in these patients had occurred [7,8].
In addition, slightly more than 10% of the patients were infected via horizontal gene transfer of class 1 integrons. Gene cassette B appeared to be highly mobile, because infections involving nearly half of strains containing type B were due to horizontal gene transfer.

This contrast with findings for gene cassette A: less than 10% of the infections involving strains containing type A were caused by integron transfer. Our data are in agreement with previously integron reservoir among drug-resistant organisms published data from Nijsen et al, who showed that 3 cases of infection (12%) resulted from horizontal gene transfer [9].

The risk of horizontal gene transfer observed in this study may be identical to that observed in a study published in 2005 [9]. Because the prevalence of integron class 1 structures has increased significantly since then, the risk of integron transfer is apparently independent of the prevalence. If there is such independence, the contribution of horizontal gene transfer to the spread of multidrug resistance in hospitals will continue to increase. Of interest, all cases of integron transmission occurred in the ICUs.

Previously, we showed that nosocomial transmission occurred in 0.7 of 10,000 patients in our hospital [5]. The cumulative incidence of integron transmission was nearly twice this value, showing that, at least in our hospital, the contribution of horizontal gene transfer to the spread of resistance is greater than that of nosocomial transmission (RR, 2.6 [95% CI, 0.9–8.1]). Knowledge about the epidemiologic characteristics of integrons is limited, but our study showed that the spread of integron-mediated antimicrobial resistance is an increasing problem. Therefore, to get additional insight about this increasing problem, there is a need for more epidemiologic studies, which will hopefully lead to actions that will prevent integron transfer in the future.
REFERENCES

Highly Resistant Gram-negative Micro-organisms: Incidence Density and Occurrence of Nosocomial Transmission (TRIANGLE study)

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Submitted
ABSTRACT

Objectives
The objectives of this study were to determine the incidence density and the occurrence of horizontal spread of Highly Resistant (HR) Gram Negative Rods (GNR) in Dutch hospitals. Also the factors that influence these outcome measures were investigated.

Methods
All patients with HR-GNR, cultured in eighteen hospitals, were included in this study during a six months period. From all available isolates the species was identified, susceptibility was determined, including the presence of Extended Spectrum B-lactamases (ESBL), and molecular typing was performed. Based on a combination of species identification, molecular typing and epidemiological data, the occurrence of nosocomial transmission was determined.

Results
The incidence density of patients with HR-GNR was 55 per 100,000 patient days (cumulative incidence was 39 per 10,000 admissions). University hospitals were statistically significant ($p=0.03$) independent determinants of a higher incidence of HR-GNR. The majority of HR-GNR were ESBL producers.

The adjusted transmission index (TI) in the participating hospitals ranged from 0.0 through 0.2. The overall adjusted TI of HR-GNR was 0.07. No determinants for a higher TI were identified.

Discussion
The nosocomial transmission rate of HR-GNR was relatively low in all hospitals, using well established transmission based precautions. The incidence density of HR-GNR was higher in University hospitals, probably related to the patient population and the complexity of the care provided.
INTRODUCTION

The increasing emergence and dissemination of Highly Resistant (HR) Gram Negative Rods (GNR) in hospitals is a world-wide problem [1]. This can be caused by patients carrying resistant pathogens on admission, by horizontal transfer between patients, by selection of resistance caused by antimicrobial use, by transfer of resistance genes between micro-organisms or by a combination of two or more of these mechanisms [2-4].

In 2005 a set of definitions for Highly Resistant Micro-organisms, including HR-GNR was published [5]. HR-GNR were defined as isolates which 1) are known to cause diseases, 2) have acquired an antimicrobial resistance pattern that hampers empirical therapy, and 3) have the potential to spread. This set of definitions is used in the Dutch National Guideline and can be used as a tool for assessing the scale of the resistance problem at a local or national level [6].

Up to now only two studies have assessed the incidence of HR-GNR in hospitals and the relative contribution of horizontal transmission [7,8]. Both were performed in a single hospital.

The objectives of this study were to determine the variations in the incidence of HR-GNR and the contribution of clonal spread in several Dutch hospitals. Secondary objectives were to determine the role of the use of antimicrobial agents, hospital characteristics and variations in infection control policies.

METHODS

Design & Setting

The study was a prospective observational multicenter study, in which five university hospitals, eight teaching hospitals and five general hospitals participated. A total of 16 microbiology laboratories were involved in the study.

Characterisation of participating hospitals

General information

For each hospital and for each Intensive Care Unit (ICU) separately, the annual number of admissions and number of patient days were registered.

Characterisation of infection control policies in participating hospitals

The project coordinator and an infection control practitioner (ICP) visited all participating hospitals and laboratories to investigate the infection control policies using a standardised questionnaire. An interview with the local ICP and microbiologist was performed.

The local infection control policies were compared with a golden standard, the national guideline to prevent nosocomial transmission of HRMO [6]. Screening policies, isolation
precautions per species, contact tracing, presence of single patient rooms and hand-hygiene procedures were included in the questionnaire.

The yearly amount of litters hand alcohol used for hand hygiene was provided. The relative use of hand alcohol was calculated by dividing the total amount of litres by the number of patient days.

Characterisation of antimicrobial policies in participating hospitals

The Defined Daily Doses (DDD) per group of antimicrobial agent, according to the ATC/DDD index 2005 from the WHO collaborating Centre for Drug statistics Methodology were obtained from each hospital in co-operation with the Dutch Working Party on Antibiotic Policy (Dutch acronym is SWAB) [9].

Hospitals who were not yet participating in the SWAB project were asked to make their antimicrobial use data available for the study.

Patient data

Patient samples were routinely collected for clinical purposes and then reanalysed for the purposes of this study. During six months, from April first until October first 2007, all first HR-GNR isolates from hospitalised patients were included. HR-GNR were defined as published previously and the criteria are summarised in table 1 [7].

Table 1: definition of Highly Resistant (HR) Gram Negative Rods (GNR)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>ESBL</th>
<th>CAR</th>
<th>QUI</th>
<th>AMG</th>
<th>CFT</th>
<th>PIP</th>
<th>TMP-SMZ</th>
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</thead>
<tbody>
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<td>B</td>
<td>B</td>
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</tr>
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<td>B</td>
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<td>A</td>
<td>B</td>
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<tr>
<td>Acinetobacter spp.</td>
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<td>Pseudomonas aeruginosa</td>
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<td>C</td>
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<td>C</td>
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<tr>
<td>Stenotrophomonas maltophilia</td>
<td>A</td>
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</tbody>
</table>

ESBL: Extended Spectrum Beta-Lactamases; CAR: carbapenems; QUI: fluoroquinolones; AMG: aminoglycosides; CFT: ceftazidim; PIP: piperacillin; TMP-SMZ: Trimethoprim-sulfamethoxazole

A: resistance against an antibacterial agent from one of the indicated groups is sufficient to define the microorganism as highly resistant
B: resistance against antibacterial agents from at least two of the indicated groups is required to define the micro-organism as highly resistant
C: resistance against antibacterial agents from at least three of the indicated groups is required to define the micro-organism as highly resistant

From each patient, in whom a HR-GNR isolate was recovered, the following variables were collected: patient information (age, sex, medical specialty and ward), date of admission and discharge, if the patient had been admitted to the ICU 30 days before the first recovery of the HR-GNR and if patients died during this admission.
Furthermore, it was registered if the HR-GNR isolate was recovered in blood samples during the admission episode and if the HR-GNR isolate was obtained from a clinical or a screening sample. A species of HR-GNR was included only once; the first time it was cultured from a patient.

**Bacterial collection**

All information and isolates were sent to a central laboratory and stored at -70°C until further testing. Before testing the isolates were subcultured twice on Columbia blood agar plates supplemented with 5% sheep blood, and grown overnight at 35-37°C. Suspensions were prepared by emulsifying bacterial isolates in 0.45% saline to the equivalent of a 0.5 McFarland turbidity standard. The same suspension was used for identification (GN) and antimicrobial susceptibility testing (AST-NO48) in the VITEK2 system (Biomerieux, Marcy l’Etoile, France). If the identification was considered ‘unacceptable’ (% probability below 85) or if the result differed from the identification from the participating hospital, API 20E/20NE (Biomerieux, Marcy l’Etoile, France) was performed as a confirmation. Suspensions for the API were made according to the manufacturer’s recommendations. Indol spot test was used as complementary test if recommended by the expert system of the VITEK2.

Susceptibility tests were interpreted according to the guidelines from the Clinical and Laboratory Standards Institute (CLSI). Presence of Extended Spectrum Beta-Lactamase (ESBL) production in all Enterobacteriaceae was determined according to the Dutch national guideline for the detection of ESBL [10]. Isolates with MIC>1 for ceftazidime (CFTZ) and/or cefotaxim (CFTX) in the VITEK2 were tested for presence of ESBL using Etest (Biomerieux, Marcy l’Etoile, France).

When Etest results were non-conclusive a disk diffusion test (Rosco diagnostica, Taastrup, Denmark) was performed using the double disk methodology. All E. coli, Klebsiella spp., Proteus mirabilis, Salmonella spp. and Shigella spp. (Group I) were tested using CFTZ and CFTX +/- clavulanic acid. All Enterobacter spp., Serratia spp., Providencia spp., Citrobacter freundii, Morganella morganii and Hafnia alvei (Group II) were tested using ceftepime (CFPM) +/- clavulanic acid. In case that the MIC in group I was out of range or if the MIC of CFTX was ≥ 16, the presence of ESBL was determined using Etest CFPM +/- clavulanic acid. Cefoxitin resistant isolates were tested for ESBL production based on synergy between CFPM +/- clavulanic acid. Klebsiella oxytoca isolates were tested for ESBL production based on synergy between CFTZ +/- clavulanic acid.

**Molecular typing of strains of the same species**

For molecular typing, chromosomal DNA was isolated using the easyMag system (Biomerieux, Marcy l’Etoile, France). Amplified Fragment Length Polymorphism (AFLP) was performed as described by Savelkoul et al [11]. Subsequent to restriction, ligation and amplification, the DNA fragments were separated on an ABI Prism 3130XL Genetic Analyser (Applied Biosystems), after which the data was analysed with the Pearson correlation coefficient and clustered by
unweighted pair-group matrix analyses using BioNumerics software, v5.10 (Applied-Maths, Sint-Martens-Latum, Belgium). Genetic relatedness was determined on basis of both visual and computerised interpretation of AFLP patterns.

Monthly and when using a new lot number a quality control was performed for the AST-NO48, GN, AST-EXN4 CARD with ATCC isolates, as recommended by the manufacturer. (Klebsiella oxytoca ATCC 700324, Pseudomonas aeruginosa ATCC 27853, E. coli ATCC 35218, 27853 and 25922, Klebsiella pneumoniae spp pneumoniae ATCC 700603).

**Definition of epidemiological linkage or ICU relatedness**

HR-GNR isolates recovered from a specimen obtained from a patient more than 48 hour after admission, or less than 48 hour after admission but shortly after a recent discharge (within 30 days) are classified as hospital-associated HR-GNR.

Epidemiological linkage was defined as two patients who had been in the same hospital ward within a maximum time window of four weeks.

Nosocomial transmission was considered present if genotypically related strains were detected in epidemiologically linked patients with a hospital-associated HR-GNR detected first at >48 hours after admission.

The Transmission Index (TI) was calculated as the number of patients with nosocomial transmission divided by the number of patients with HR-GNR not acquired by nosocomial transmission (potential index cases). The TI in each participating centre was adjusted for the proportion of isolates which were not available, Adjusted TI = TI x (total number of isolates/number of isolates available for typing).

HR-GNR isolates recovered from a patient who was on an ICU, or who had been on an ICU during 30 days prior to the recovery of the isolate, were classified as “ICU-associated”.

**Data-analyses, quality control & statistics**

Privacy of patients was provided by coding all data according to the requirements of the National Privacy Regulations in The Netherlands. Medical ethic review was not required for this study according to the Dutch law. The data were entered in a database and analysed using Excel and the Statistical Package for Social Sciences software (SPSS version 16).

Categorical variables were analysed by Fisher’s exact test or the chi-square test when appropriate, and continuous variables were analysed using a t test or the Mann-Whitney U test when appropriate. A linear regression analysis was performed to adjust for confounding. Statistical significance was accepted when the chance for coincidence was less than 5%.
RESULTS

In this study five university hospitals, eight teaching hospitals and five general hospitals participated. On average there were 227,596 (SD=55,162) patient days and 28,270 (SD=5,389) admissions per year in the university hospitals, 169,262 (SD=6,857) patient days and 25,669 (SD=6,857) admission per year in the teaching hospitals and 76,417 (SD=1,396) patient days and 12,011 (SD=1,396) admissions per year in the general hospitals (data from 2007). The annual mean length of stay was 7.9 (SD=0.6) days in the university hospitals, 6.6 (SD=0.4) days in teaching hospitals and 6.4 (SD=0.3) days in general hospitals.

During the 6 months study period a total of 892 HR-GNR were recovered in 786 patients (Table 2) from clinical cultures and screening cultures all together. The mean age of these patients was 54.5 years (SD=24), the mean time between admission and detection of HR-GNR was 16.2 days (SD=9.2) and the mean length of hospital stay was 37.2 days (SD=46.1).

During hospitalisation, 132 of the patients (16.8%) died. In the 30 days preceding the first isolation of a HR-GNR, 329 patients (41.9%) had been admitted on the ICU. In 95 patients (10.7%) the HR-GNR was isolated from blood cultures. In 616 patients (69.0%) the first HR-GNR was recovered more than 48 hours after admission.

From the total of 892 HR-GNR isolates, 675 (75%) were available for further analyses (not all isolates were saved and frozen in the participated hospitals). This collection consisted of 579 Enterobacteriaceae: E. coli, 305 isolates (57.4% ESBL producing); Klebsiella species, 113 isolates (73.5% ESBL producing); Enterobacter species, 76 isolates (61.8% ESBL producing); Citrobacter species, 28 isolates (21.4% ESBL producing); Proteus mirabilis, 37 isolates (5.4% ESBL producing); Morganella morganii, 9 isolates; Serratia marcescens, 5 isolates; Providencia species, 2 isolates; Salmonella species, 2 isolates; Shigella species, 2 isolates. Furthermore the collection consisted of 96 Glucose non-fermenting GNR: Pseudomonas species, 39 isolates; Stenotrophomonas species, 26 isolates; Achromobacter species, 6 isolates; Acinetobacter species, 15 isolates; chrysomomas spp. 8 isolates; Ochrobactrum anthropi 1 isolate; Sphingomonas paucimobilis, 1 isolate.

Of the Enterobacteriaceae, 2 isolates (0.3%), 1 E. cloacae isolate and 1 E. coli isolate, were resistant to imipenem (IMI), 202 isolates (35.0%) were resistant to tobramycin (TOBR) and 295 (51.0%) were resistant to ciprofloxacin (CIP). From the Glucose non-fermenting GNR, 45 isolates (64.3%) were resistant to CFTZ, 29 isolates (41.1%) were resistant to IMI, 32 isolates (45.7%) were resistant to TOBR and 44 isolates (62.9%) were resistant to CIP. None of the isolates was resistant to all drugs that had been tested (pan-resistant).

Screening and infection control policies in the participating hospitals

There were considerable variations in the frequency of sampling for clinical cultures, as well as standardised sampling for screening cultures between the participating hospitals. Four
<table>
<thead>
<tr>
<th>Hospitals</th>
<th>HR-GNR(^1)</th>
<th>Patients with HR-GNR(^1)</th>
<th>Mean Age</th>
<th>Mean L.O.S.(^2)</th>
<th>Mean time between admission and detection</th>
<th>Died during admission</th>
<th>Admission on ICU(^3)</th>
<th>Cultured in blood-samples</th>
<th>Cultured in screening</th>
<th>Incidence Density per 100,000 days</th>
<th>Transmission Index per 10,000 Adm(^4)</th>
<th>Adjusted Transmission Index</th>
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<td>1</td>
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<td>42.8</td>
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<td>18.5</td>
<td>16 (17.0%)</td>
<td>34 (36.2%)</td>
<td>10 (10.2%)</td>
<td>9 (9.2%)</td>
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<td>64 (50.0%)</td>
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<td>25.9</td>
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<td>24 (42.1%)</td>
<td>5 (6.8%)</td>
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<td>43 (34.7%)</td>
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<td>4</td>
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<td>49.0</td>
<td>27.0</td>
<td>1 (25.0%)</td>
<td>2 (50.0%)</td>
<td>0</td>
<td>1 (25.0%)</td>
<td>11.1</td>
<td>6.7</td>
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</tr>
<tr>
<td>17</td>
<td>4</td>
<td>4</td>
<td>67.3</td>
<td>23.3</td>
<td>8.3</td>
<td>1 (25.0%)</td>
<td>0</td>
<td>2 (50.0%)</td>
<td>0</td>
<td>11.2</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>5</td>
<td>48.6</td>
<td>57.4</td>
<td>44.7</td>
<td>2 (40.0%)</td>
<td>2 (40.0%)</td>
<td>0</td>
<td>0</td>
<td>14.6</td>
<td>8.9</td>
<td>0</td>
</tr>
<tr>
<td>total</td>
<td>892</td>
<td>786</td>
<td>54.5</td>
<td>37.2</td>
<td>16</td>
<td>132 (16.8%)</td>
<td>329 (41.9%)</td>
<td>95 (10.7%)</td>
<td>271 (30.4%)</td>
<td>55</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

HR-GNR\(^1\) = Highly Resistant Gram Negative Rods; L.O.S.\(^2\) = Length Of Stay; ICU\(^3\) = Intensive Care Unit; Adm\(^4\) = Admission
hospitals did not perform active screening for HR-GNR on admission to the ICU when patients had been in a foreign hospital for more than 24 hour and within 2 months before admission [6]. In this case, 9 hospitals did not perform active screening for HR-GNR upon admission on a non-ICU ward. Fifteen hospitals cultured sputum and rectal swabs routinely from all patients on the ICU once or twice a week. One of the hospitals performed an additional monthly screening for HR-GNR in all ICU patients (rectal swab in a broth enrichment containing tobramycin) and one hospital performed an additional weekly screening for ESBL producing isolates in all ICU patients (rectal swab plated on a selective ESBL agar plate).

Contact tracing after an unexpected finding in the ICU was ‘always’ performed in 3 hospitals, ‘sometimes’ in 11 hospitals. And 4 hospitals ‘never’ performed contact tracing after an unexpected finding. In non-ICU wards, 2 hospitals ‘always’ performed contact tracing after an unexpected finding, 11 hospitals ‘sometimes’, and 5 hospitals ‘never’. Table 2 shows the proportion of HR-GNR isolated from screening cultures (contact tracing & screening after admission in foreign country).

Once a HR-GNR was detected the following infection control policies were followed. In all hospitals, patients with ESBL producing HR-GNR isolates were placed in contact isolation on the ICU unit. Patients with non-ESBL producing HR-GNR on the ICU were placed in contact isolation in 18 of the 19 hospitals. On non-ICU wards more variation in isolation precautions was found. Nine hospitals performed contact isolation ‘always’ in a single room and 9 hospitals used a single room only when available on the ward. Isolation measures of patients with HR-GNR were abandoned in all hospitals after 2 or more negative cultures. Five hospitals used an alert system for patients with HR-GNR (alert when patients with HR-GNR were readmitted to the hospital).

The use of alcohol used for hand hygiene per patient day varied considerably between the hospitals. A strong and significant correlation was observed between the amount of hand alcohol use and the proportion of ICU days on the total number of hospital days (Figure 1).
Figure 1: Amount of alcohol used for hand hygiene (litre alcohol per 100 patient days) versus the ratio of the number of ICU-days / total number of patient days

**Antimicrobial use**

The mean total annual use of antimicrobial agents in the university hospitals was 64 Defined Daily Doses (DDD) per 100 patient days (SD=10) and 54 DDD/100 patient days in the teaching hospitals (SD=8). The annual use in the general hospitals was not available from all hospitals or it included the antimicrobial use from nursing homes, and could therefore not be used for the analysis. Difference in annual use between the group of university- and the teaching hospitals was not significant, as shown in Figure 2.

Figure 2: Annual antimicrobial use in university and teaching hospitals in DDD/100 patient days
Incidence density of HR-GNR
The mean cumulative incidence of patients with HR-GNR was 39 per 10,000 admissions and the mean incidence density was 55 patients with HR-GNR per 100,000 patient days. The mean cumulative incidence of HR-GNR in the university, teaching and general hospitals is shown in Figure 3. In the univariate analyses the incidence density was statistically significant associated with the proportion of ICU days in a hospital (p<0.001), with the type of hospital (university, teaching or general) (p=0.007) and with the annual amount of antimicrobial use (p=0.031). Linear regression analysis showed that University hospitals were the only statistically significant (p=0.03) independent determinant of a higher incidence of HR-GNR.

Transmission of HR-GNR
Results from molecular typing showed no clusters in 6 hospitals. In the other hospitals multiple clusters were found (2 clusters in 3 hospitals, 3 clusters in 1 hospital, 7 clusters in 3 hospitals, 8 clusters in 3 hospitals, 9 clusters in 1 hospital and 11 clusters in 1 hospital). The number of patients within one cluster varied from 2 to 11 (modus = 2). Clusters involving patients from different hospitals were not investigated.

Combining the results of typing with the epidemiological information resulted in 22 clusters with epidemiological linkage (11 involved transmission on the ICU). The adjusted TI in the participating hospitals ranged from 0.0 through 0.2 as shown in Table 2. The number of patients due to nosocomial transmission per index case varied from 0 through 6 per hospital. In the general hospitals no transmission was observed. In the university and teaching hospitals the adjusted TI were comparable, Figure 4).
Chapter 3.4

Figure 4: Adjusted Transmission Index of highly resistant Gram negative rods (HR-GNR) in university and teaching hospitals

For the analyses of risk factors for nosocomial transmission the hospitals with less than 10 HR-GNR available for typing, were not taken in account. There was no relation between the incidence density and the adjusted TI. Hospital size, as indicated by the annual number of patient days and the annual number of admissions, had no significant relation with the TI. The TI tended to be higher in the teaching hospitals; however this relation was not statistically significant. Also the relative size of the ICU and the design of the ICU ward (proportion of single patient rooms on the total number of ICU beds) had no relation with the TI. Furthermore no relation between TI and the amount of antimicrobial use in DDD per 100 patient days was found.

Infection control precautions, as variation in isolation precautions, the performance of contact tracing, active screening, and amount of alcohol used for hand hygiene and alert systems did not show a relation with the TI. Also hospitals with an active screening policy were not associated with a higher TI. Multivariate analysis did not reveal a determinant that was associated with a higher TI.

DISCUSSION

In this multicenter study we determined the incidence density of HR-GNR and the role of nosocomial transmission. There were large variations in the incidence density (Range: 7 – 123 per 100,000 patient days). The mean incidence density was 55 per 100,000 patient days. The cumulative incidence was 39 per 10,000 admissions.

This was comparable to a previous report from a single centre study in a University hospital in Germany by Vonberg et al. (incidence density of 43 per 100,000 patient days) [8]. The definitions of HR-GNR used in that study were slightly different from ours. Multidrug-resistant GNR
were defined as Gram-negative aerobic rods that were susceptible to fewer than 2 groups of bactericidal antimicrobial agents. Our definitions were based upon both single-drug resistance (carbapenem), presence of resistance mechanisms (e.g. ESBL) or multidrug resistance, depending on the kind of species and the impact on clinical decision making, which will probably result in a higher rate in our study as more GNR will be included. The difference in definitions used in various studies makes it difficult to compare the incidence of HR-GNR between hospitals and countries. For future benchmarking it is important to accept a standardised set of definitions. The definitions used in this study are useful for this purpose [5].

The most frequently isolated species was *E. coli*, followed by *Klebsiella* species and *Enterobacter* species. More than half of the isolates (54.3%) were ESBL producers, illustrating the rapid increase of this resistance mechanism in recent years. Similar as reported in a Canadian study, ESBL-producing *E. coli* bacteria have become more common than ESBL-producing *Klebsiella* spp [12].

There are several indications that ESBL is becoming more prevalent in the community and is introduced in the hospitals from the community. The appearance of ESBL in the food chain (especially in poultry) is considered to contribute to the emergence of ESBL in the community [13].

Although the use of carbapenems is increasing in the Netherlands, it is still very low (0.8 DDD/100 patient days in 2007), compared to other European countries, which may explain the extremely low rate of carbapenem resistance observed in this study [14].

The patients colonised or infected with HR-GNR had a relatively long length of stay in the hospital (mean=37.2 days), which was also found by Vonberg et al [8]. The mean length of stay of the entire population involved in this study was 6 to 7 days. It is well known that the detection of highly resistant micro-organisms is associated with a longer length of stay [15,16].

There are two explanations for this. First, a prolonged length of stay poses an increased risk for cross-infection. Second, a longer stay also increases the likelihood that a resistant micro-organism, that was carried upon admission, is detected in routine diagnostics. This higher detection chance is explained by the likelihood that a patient is cultured and by the chance that a resistant micro-organism is detected in a culture that has been taken.

Resistant micro-organisms are often present in very small amounts, compared to a huge amount of susceptible micro-organisms. Antimicrobial use during hospitalisation may offer a selective advantage for multiplication of the resistant strains, which subsequently increases the likelihood that they are detected in microbiological samples.

The extremely long length of stay for patients with HR-GNR is also indicative that these patients suffer from more severe underlying disease. This is also reflected by the high mortality in this group. More than 16% died during the admission. In univariate analyses the incidence
density of HR-GNR was statistically significant associated with the type of hospital, the proportion of ICU days and the amount of antimicrobial use.

The importance of the ICU ward may be biased by the intensified screening that is carried out by many ICU wards, resulting in a higher chance for the detection of HR-GNR. The screening policies within the hospitals were not standardised in this study and the questionnaire revealed the variations in the strategies. To assess the possible effect of screening on the observed incidence density and TI we evaluated the association of these outcome measures with the proportion of HR-GNR that was detected in screening samples. No association was found. We therefore conclude that the effect of screening strategies has not influenced the results significantly.

In a single centre study, in a teaching hospital, the ICU ward was identified as the main determinant for the presence of HR-GNR in a previous study [7]. This was also found in hospitals participating in our study in univariate analysis. However, after multivariate analysis the ICU was not significant.

The only independent variable that was associated with the incidence density of HR-GNR were the University hospitals, which had the highest incidence of HR-GNR (p=0.03). The reason for this is probably that the complexity of the patient population in combination with the complexity of the care provided, are the main determinants for the observed incidence of HR-GNR. This is also in line with the long length of hospital stay and high mortality of patients with HR-GNR. Since we have not obtained data on the severity of disease of the individual patients we cannot confirm this hypothesis.

Antimicrobial use was not identified as determinant of HR-GNR. A restriction of our study is the fact that we only investigated antimicrobial use at the hospital level. Since 40% of patients with HR-GNR had been admitted to an ICU department, a possible association of incidence density of HR-GNR with local antimicrobial use at the ICU level can not be excluded.

Horizontal spread of HR-GNR may contribute to a high incidence of HR-GNR as well. We used AFLP to determine the genetic relatedness of all strains of the same species [7]. The analysis of AFLP data showed that the majority (66%) of HR-GNR isolates were unique. Comparison of AFLP results with epidemiological data, within the individual hospitals, revealed a limited number of epidemiologically linked HR-GNR.

In general the number of secondary cases within clusters was small. In the largest cluster 6 patients were involved. This cluster was found in a hospital that was aware of an outbreak at that time. The overall transmission index was 0.06 (adjusted TI = 0.07). There are hardly any studies to compare these data with. Only the study of Vonberg et al. describes an acquisition rate of multidrug resistant GNR of 4.7%, which is within the range of our findings [8]. Most other reports that provide data on transmission are descriptions in outbreak situations and are therefore not comparable to our findings.
We determined the contribution of various infection control measures, the antimicrobial policy and hospital characteristics to the occurrence of nosocomial transmission of HR-GNR. No independent variable was identified. This may have several explanations.

First, the number of cases of nosocomial transmission was relatively low in almost all hospitals, which limits the power of the study. Second, there was strong association between several parameters that are likely to be important but have opposite effects on the TI. For example, the amount of hand alcohol used is presumed to be associated with a reduction of horizontal spread in the hospital [17].

On the other hand, ICU’s are known for the relative high level of nosocomial spread [7]. Furthermore the proportion of ICU beds is considerably higher in the university hospitals than in the teaching and general hospitals. Figure 1 clearly shows that there is a strong and significant association between the amount of hand alcohol use and the proportion of ICU days. This means that hospitals with a large proportion of ICU beds and therefore more likely to have a high TI of HR-GNR also use more hand alcohol which may lower the TI. This makes it difficult to judge the contribution of hand alcohol to the prevention of nosocomial spread of HR-GNR.

Finally, it is unlikely that individual infection control measures have a large effect on the TI. Recent studies suggest that successful infection control programs use a number of control measures concomitantly, also called a bundle [18]. In the hospitals participating in this survey nosocomial transmission of HR-GNR was controlled effectively using a guideline with relatively simple contact precautions [5].

In all hospitals the TI (and the adjusted TI) was far from the breakpoint of ongoing transmission (ie, TI greater than 1.0). The variations of the TI between hospitals could not be explained by the variables that we studied. The number of HR-GNR that were related to horizontal spread in the hospital were only a minority of all HR-GNR found. Therefore, we conclude that the majority of HR-GNR were probably carried upon admission by the patients and detected during the course of hospitalisation.

Our study provides useful information about the incidence of HR-GNR in Dutch hospitals using a well defined set of criteria. Other hospitals can use these data as a benchmark for their own situation. Most HR-GNR were ESBL producers, indicating the rapid increase of this resistance mechanism in recent years. The transmission of HR-GNR in the participating hospital was effectively controlled using well established transmission based precautions. The only independent determinants of the incidence of HR-GNR were University Hospitals, which is probably a surrogate marker for the complexity of the patient population and the complexity of the care provided.
ACKNOWLEDGMENTS

We thank the infection control practitioners, laboratory site staff, pharmacists and microbiologist at all hospital/laboratory that participated in the study. We thank the Working party on Antibiotic Policy (SWAB) for providing the antimicrobial use data from the participating hospitals.
REFERENCES


Targeted interventions to improve antimicrobial use in hospitals
A Standardised Protocol for Perioperative Antibiotic Prophylaxis is associated with Improvement of Timing and Reduction in Costs

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ABSTRACT

The objectives of this study were to implement a uniform guideline for perioperative antibiotic prophylaxis (PAP) and to measure the impact on timing and costs of PAP.

The effects of implementation of the new guideline describing the application of PAP were measured by comparing the choice of agents and their timing in a random sample of procedures before and after implementation.

Before the intervention, 153 procedures from different specialties were observed; eight different antibiotics in different dosages were used and in 20% of the procedures, PAP was given after the incision. Two months after the intervention, 147 procedures were observed; three different antibiotics were given and all were used in the correct dosage; there was a significant reduction of administration of PAP after the incision from 20 to 7% (p = 0.002).

Besides the quality improvements, the modified PAP protocol resulted in a net annual savings of at least US$112,000. This study shows that the implementation of a uniform, simple and clear protocol for PAP was associated with an improved dosing and timing. The costs of PAP were also reduced.
INTRODUCTION

Perioperative antibiotic prophylaxis (PAP) is a proven strategy to prevent surgical site infections [1]. However, the effectiveness of PAP is dependent on appropriate administration to patients undergoing surgical procedures in which it is indicated.

The optimum agent, which is both safe and effective, should be selected and the correct dose should be administered at the right time. In prolonged procedures, re-dosing may be needed to maintain effective serum and tissue levels throughout the operation. This principle is widely accepted and noted in several guidelines [2-4]. Unfortunately, adherence to guidelines is often poor. For example, in a national retrospective cohort study with medical record review in the USA it was found that in the majority of hospitals adherence was cumbersome [5].

The aim of our study was to implement a uniform guideline for PAP and to measure the impact on adherence, timing and costs.

METHODS

Hospital setting

The Amphia Hospital was founded in 2001 after a merger of three hospitals. It is a 1370-bed teaching hospital in which all medical specialties are available. In 2005 there were 40,525 admissions and 265,665 bed-days. There are three operating room complexes, in which approximately 11,000 surgical procedures are performed annually. The implementation of the new guideline for PAP took place in January 2006.

PAP guideline

Before the intervention there were several different guidelines in use, mainly originating from the three hospitals before the merger. For example, the prescribed PAP for a total hip procedure in hospital A was co-amoxiclavulanic acid, in hospital B it was cefamandole, and in hospital C it was flucloxacillin.

In the new guideline cefazolin was the first choice for all procedures. If anaerobic coverage was needed, metronidazole was added [6]. Clindamycin was preferred as an alternative agent for patients reporting an allergy to β-lactams. Except for procedures using implants, a single preoperative dose of PAP was recommended [7-9].

The new guideline was primarily developed by the antibiotic policy committee, consisting of consultant microbiologists and hospital pharmacists and was approved by all surgeons and anaesthetists. Subsequently, the guideline was distributed within the hospital. It included the antibiotic agent, the recommended dosage and information about PAP for each type of surgical procedure.
Before the implementation of the new guideline, all nurses, pharmacy assistants and anaesthesia technicians who were involved in PAP were instructed by the project coordinator. The weekend before the actual start of the new guideline, all antimicrobials in supply closets were switched in the operating rooms and on the wards.

**Follow-up assessment**

Before and after the implementation of the new PAP guideline, the application was measured on a random sample of procedures on all three operating room complexes. The personnel on the operating room complex were unaware of the objective of the measurements.

The following variables were collected: type of procedure, medical specialty, surgeon, operating room number, type of antimicrobial agent, dosage of antimicrobial agent, time of administration of first dose, time of incision. In the case of a surgical procedure under blood occlusion, the moment of blood emptiness was registered as time of incision.

In caesarean section procedures, PAP was administered directly after the umbilical cord had been clamped, according to national and international guidelines [10]. These procedures were excluded from the analyses on timing because PAP was deliberately administered after incision.

The registrations took place approximately two months before and two months after the implementation of the new guideline. The costs for antimicrobial agents in the operation complexes in 2006 were compared with the cost in 2005. Total acquisition costs of cefamandole, cefazolin and co-amoxiclav (dosage 2200 mg) were calculated. These agents were used exclusively for PAP.

**Data analyses**

Data were entered in a database, and analysed using the Statistical Package for Social Sciences software (SPSS, version 12.0). The effectiveness of the new PAP guideline was judged by comparing the antimicrobial agents used, the dosage and the timing before and after the implementation. Categorical variables were analysed by Fisher’s exact test or the Chi-squared test. Statistical significance was accepted when the chance for coincidence was $p<0.05$.

**RESULTS**

Before the implementation of the new guideline, 153 procedures from different specialties were observed. Table I shows that eight different antimicrobial agents were used. In five cases there was no information about the dosage, and in 17 cases antimicrobial agents were administered in a dosage other than recommended. To analyse the timing of administration, 15 procedures were excluded: three caesarean sections and 12 procedures for which time of administration was not available. From the 138 procedures available for analysis of timing of administration, on 28 occasions (20%) PAP was administered after the incision (Figure 1).
Two months after the implementation of the new guideline, 147 procedures from different specialties were observed. Three different antimicrobial agents were used; cefazolin in 135 (91.8%), cefazolin in combination with metronidazole in 11 (7.5%), and cefuroxime in one (0.7%) (Table 1). In all of these procedures the antimicrobial agents were administered in the recommended dosage, which was a significant improvement (p<0.001).

Table 1: Use of antimicrobial agents for perioperative antibiotic prophylaxis before and after implementation of the new guideline

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Before new guideline</th>
<th>After new guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Co-amoxiclavulanic acid</td>
<td>70 (45.8)</td>
<td>-</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>34 (22.2)</td>
<td>-</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>24 (15.7)</td>
<td>135 (91.8)</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>16 (10.5)</td>
<td>-</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>4 (2.6)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Cefuroxime+metronidazole</td>
<td>3 (2.0)</td>
<td>-</td>
</tr>
<tr>
<td>Cefazolin + metronidazole</td>
<td>-</td>
<td>11 (7.5)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>1 (0.7)</td>
<td>-</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>1 (0.7)</td>
<td>-</td>
</tr>
<tr>
<td>Total procedures</td>
<td>153 (100)</td>
<td>147 (100)</td>
</tr>
</tbody>
</table>

To analyse the timing of administration, 140 procedures were included (seven caesarean sections were excluded). In 10 procedures (7%) PAP was administered after the incision (Figure 1).
1). The improvement of timing, expressed as the likelihood of administration of PAP after the incision, was statistically significant (RR: 0.35, 95% CI: 0.18-0.68; \( p = 0.002 \)).

Before and after intervention, 40 procedures with blood occlusion of the incision site were judged. In three of these 40 procedures the antimicrobial agents were administered after applying the tourniquet. In the 37 remaining procedures, antimicrobial agents were administered before applying the tourniquet. In 38% of these procedures, PAP was administered within 5 min before applying the tourniquet. Considering the application of the tourniquet as time of incision, procedures with blood occlusion are a risk factor for late administration of PAP (within 5 min) compared to timing of PAP in procedures without blood occlusion (RR: 2.78, 95% CI: 1.6-4.6; \( p = 0.002 \)).

Before the intervention, co-amoxycillin and cefamandole were most frequently used for PAP. The switch from cefamandole to cefazolin resulted in an estimated annual saving of US$90,500. The switch from co-amoxycillin to cefazolin saved US$21,500 per year. For the other agents that were used for PAP before the intervention it was not possible to provide a reliable estimate on the savings. As cefazolin was cheaper than all other agents involved, the total costs will have been further reduced but because these agents were also used for therapy in other areas of the hospital we could not calculate these savings. Therefore, the total annual savings associated with the implementation were at least US$112,000.

**DISCUSSION**

The implementation of a new guideline for PAP was associated with several beneficial effects. There were statistically significant improvements considering both dosing and timing and the costs of PAP were reduced. There were several factors which contributed to a successful implementation.

First, the new guideline was founded on evidence-based international guidelines, which were translated to the local situation by the antibiotic policy committee. All surgical specialties and anaesthetists were then asked for their comments. In this way the guideline was fully accepted by the clinicians.

The guideline in itself was a relatively simple schedule consisting of only three different agents. When the guideline was accepted, all healthcare workers involved in the administration of PAP were instructed by a dedicated person, the project co-ordinator. It is likely that the simplicity of the guideline, combined with personal instruction, were critical success factors for the implementation. In addition, the simplicity of the protocol and reduction of the number of agents used resulted in the notable improvements in dosing and timing. These aspects had not been explicit parts of the instruction.
In a study by Classens et al., 28% of patients had PAP after the incision [11]. A more recent multicentre study of van Kasteren et al. showed that between 20 and 30% of patients received PAP after the incision [12]. In our study, before the intervention the percentage of patients receiving PAP after the incision was similar to these other studies (20%). The intervention was associated with a major improvement in timing, with only 7% administered after the incision, although this had not been an explicit part of the intervention.

There are several plausible reasons for this effect. Administration after the incision was mainly caused by the short time the patient was in the operating room complex before the surgical procedure started. With the increasing pressure on budgets, hospitals have markedly improved efficiency and productivity over recent years. In the process of increasing efficiency, less time remains for the preoperative preparation of the patient. As shown in Figure 1, approximately half of the patients received PAP within 15 min before the incision. Given this short time-frame, a small delay will easily result in administration after the incision. With the new protocol, the healthcare workers involved in the administration of PAP could act based on the protocol. Before the intervention, anaesthesia technicians often had to wait for the surgeon to decide whether PAP was indicated and which agent was preferred. This may have resulted in a delay in the administration of PAP. With the introduction of a simple protocol, including the explicit description of the indications for PAP, the occurrence of fewer delays would be the most likely explanation for the improvement in timing.

The importance of timing has been demonstrated in many studies [4,6,13]. Administration of the initial dose of PAP after the incision is associated with a 2-6-fold higher surgical site infection rate [10,14]. Compared with other studies, the adherence to the new guideline for PAP is relatively good, but there is still significant room for further improvement. To achieve a continuous high adherence to guidelines for PAP a system of intense surveillance that is able to provide monitoring, analysis of variation, assessment of interventions, feedback and education is required [4].

In conclusion, this project shows that a uniform, simple and clear protocol combined with personal instruction improves not only the choice and dosage of antibiotics but also the timing. Furthermore, the switch to cefazolin resulted in a net saving of at least US$112,000 per year.

**ACKNOWLEDGEMENTS**

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REFERENCES


Improving Antimicrobial Use in the Hospital: Targeting Fluoroquinolones Using Multiple Interventions

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ABSTRACT

Objectives
To determine the effects of multiple targeted interventions on the use of fluoroquinolones and observed resistance to fluoroquinolones in *Escherichia coli* in hospitalised patients.

Methods
A bundle consisting of four interventions to improve the use of fluoroquinolones was implemented. The outcome was measured using the monthly use of intravenous (IV) and oral fluoroquinolones and the susceptibility patterns for *E. coli* from hospitalised patients. Statistical analyses were performed using segmented regression analysis and segmented Poisson regression models.

Results
Before the bundle was implemented, the annual use of fluoroquinolones was 2.7 DDD/100 patient days. After the interventions, in 2007, this was reduced to 1.7 DDD/100 patient days. The first intervention, a switch from IV to oral medication, was associated with a stepwise reduction of IV fluoroquinolone use of 71 prescribed daily doses (PDD) per month (95% Confidence Interval (CI): 47-95, *p*<0.001). Intervention two, introduction of a new antibiotic guideline and education program, was associated with a stepwise reduction of overall use of fluoroquinolones (reduction: 107 PDD/month (95%CI: 58-156).

Before the interventions the fluoroquinolone resistance rate was increasing on average by 4.6% (95% CI: 2.6-6.1) per year. This increase levelled off, which was associated with intervention two and intervention four, active monitoring and feedback of prescriptions. Trends in resistance to other antimicrobial agents did not change.

Conclusions
This study showed that the hospital-wide use of fluoroquinolones can be significantly reduced by an active policy consisting of multiple interventions. There was also a stepwise reduction in fluoroquinolone resistance associated with the bundle of interventions.
INTRODUCTION

The use of antimicrobial agents and rates of antimicrobial resistance vary significantly between countries [1-4]. A substantial proportion of the antimicrobial use is considered inappropriate [5]. Apart from the unnecessary costs and potential harm to the patient, inappropriate use can lead to increased selection for and transmission of resistant micro-organisms.

A recent survey in our hospital showed that approximately 40% of all antibiotic prescriptions were considered inappropriate (e.g. unnecessary, incorrect choice, or incorrect dosage). The only independent variable associated with inappropriate use was the use of fluoroquinolones [5]. In many cases the use of fluoroquinolones was incorrect because there was no indication for antimicrobial therapy, alternative antimicrobials should have been used (based on hospital and (inter)national guidelines) or fluoroquinolones were used intravenously where oral forms would suffice.

The use of fluoroquinolones promotes the spread of antibiotic resistance genes by activating an SOS response, as reported by Beaber et al [6]. This means that the use of fluoroquinolones could account for the rapid manner in which resistance genes are disseminating. We therefore performed an intervention study to correct the use of fluoroquinolones in hospitalised patients and to determine the effect on associated costs and the observed resistance in *Escherichia coli*.

METHOD & MATERIALS

The study was designed as a prospective interrupted time series study consisting of 4 interventions. The study was performed in the Amphia hospital which is a 1370 bed teaching hospital including most medical specialties. The out-patient clinic, the intensive care unit (ICU) and the psychiatry ward were excluded from the interventions. However, these departments were included in the analysis for observed resistance. In 2006 there were 41,712 admissions and 279,403 bed-days.

The interventions

During the study period four interventions involving the use of ciprofloxacin (CIP), the only fluoroquinolone used in the Amphia hospital, were carried out. The interventions were coordinated by a single dedicated project coordinator. The project coordinator coordinated all activities of co-workers involved in the project, i.e. consultant microbiologists, pharmacists, pharmacy-assistants and medical specialists.
Intervention 1: switch from intravenous (IV) to oral medication (SWITCH project)  
(period: January first 2006 until December 31st, 2007)  
The guideline for an early switch was developed primarily by the hospital antibiotic policy committee and was subsequently approved by the physicians in the hospital. The criteria for a switch from IV to oral were: first, the patient had to be able to take oral medication; second, the patient had to be hemodynamically stable (pulse <100 beats/min and systolic blood pressure >100 mmHg) and third, no switch was allowed if the patient was on parenteral nutrition [7-10].  
If a switch was performed, the following rules were applied: a dosage of 200 mg bid IV was switched to 500 mg bid po and 400 mg bid IV was switched to 750 mg bid po. The use of aluminium- or magnesium- containing antacids, sucralfate, calcium-, bismuth-, zinc- and iron salts disturbs the uptake of CIP [11]. Therefore patients taking such medication, took CIP oral medication more than 2 hours before or greater than 4 hours after the other medication.  
From Monday through Friday all patients with CIP IV prescriptions were identified in the pharmacy department information system and checked against the hospital antibiotic policy by a pharmacy assistant. If the patient was considered suitable for a switch, the attending physician was contacted by the pharmacy assistant and a switch was recommended. If the patient was considered potentially suitable but no definite conclusions could be made, the consultant microbiologist was contacted by the pharmacy assistant for advice. If the consultant microbiologist considered the patient suitable for a switch he contacted the attending physician and a switch was recommended. If the patient was considered not to be suitable for a switch, the IV therapy was continued.

Intervention 2: New antimicrobial guideline and educational program  
On May 15th, 2006 a new guideline for antibiotic use was issued in the Amphia hospital. This guideline was developed by the hospital antibiotic policy committee and subsequently approved by the physicians. The guideline was based on national and international recommendations and adapted to local susceptibility patterns of pathogens [12]. The guideline recommended that empiric use of CIP would be reserved for patients with severe gastro-enteritis, prostatitis or diabetic foot ulcers. In other cases the use of CIP should be based on microbiological diagnostic results.  
The guideline was sent to all interns, residents and physicians in a pocket-sized brochure and was made available on the intranet of the Amphia Hospital. In June 2006, residents attended group education sessions informing them about the prescription of antimicrobial agents for clinical patients. Restrictive use of CIP was highlighted.

Intervention 3: Restriction note on laboratory reports  
In November 2006 a comment on the use of CIP was added to all microbiology results reports: “According to the hospital guideline on antimicrobial treatment, ciprofloxacin is considered a restricted antibiotic which should be prescribed on strict indications only”. Furthermore, a
letter was sent to all physicians about the increasing resistance for CIP in the Amphia hospital, requesting them to follow the local guidelines for antimicrobial therapy.

**Intervention 4: Active monitoring of prescriptions and feedback**

On January 1st 2007 the fourth intervention was initiated. This consisted of active monitoring of CIP prescriptions and providing feedback to the prescriber. The hospital’s computerised pharmacy records were used to retrieve all orders for CIP prescriptions on a daily basis. Initial screening for appropriateness of the prescriptions was performed by the project coordinator according to the local antibiotic prescription guidelines. If prescriptions did not meet the criteria specified in the guideline, the consultant microbiologist would contact the prescribing intern/resident to discuss the appropriateness of CIP use. The recommendations were registered on a standardised form and follow up of the recommendations was checked by the pharmacy assistant.

**Outcome measures**

The monthly use of CIP IV and oral in grams was calculated based on the pharmacy department data. Treatment duration was calculated as the difference (in days) between the prescription start and stop dates. If the dosage or method of administration (IV or oral) of CIP was changed during therapy, it was assumed the change had occurred at the beginning of the first day the patient received the new preparation. The use of CIP (IV, oral and total) was translated into Prescribed Daily Doses (PDD) using 0.8 g as 1 PDD for IV and 1.0 g as 1 PDD for oral CIP.

The CIP use data were evaluated monthly in a team, including a hospital pharmacist, pharmacy assistant, consultant microbiologist, and the project coordinator. The annual total antibiotic consumption and CIP consumption were translated to PDD and Defined Daily Doses (DDD) according to the ATC/DDD index 2005 from the WHO Collaborating Centre for Drug statistics Methodology [13]. Consumption data from the out-patient department were excluded from the analysis.

Susceptibility patterns for *E. coli* including CIP, cefuroxim (CFRX), ceftazidim (CFTZ), trimethoprim sulfamethoxazole (TMP-SMZ) and tobramycin (TOBR) from hospitalised patients, recovered after more than 48 hour after admission, were analysed. Susceptibility patterns were obtained from the laboratory information system from January first 2004 up to December 31st, 2007.

Antimicrobial susceptibility testing was performed using an automated system (Vitek Biomérieux). Interpretation of the antimicrobial susceptibility test results was based on guidelines from the Clinical and Laboratory Standard Institute (CLSI) [14]. Repeat isolates from a patient after recovery of the initial isolate were excluded from analysis, unless there was a major difference in susceptibility pattern. A major difference was defined when at least one change from susceptible to resistance was observed. Analyses were performed considering intermediate susceptibility as susceptible.
Chapter 4.2

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Targets and funding

At the initiation of the interventions, the following targets were defined: 1) a 50% reduction of IV CIP prescriptions; and 2) a 30% reduction of the absolute amount of CIP use.

Based on these assumptions and the anticipated cost savings, the hospital management funded the project by financing a study-coordinator (12 hours per week) and a pharmacy assistant (18 hours per week) during 2006 and 2007.

Data analysis

Privacy of patients was maintained by coding all data according to the requirements of the privacy regulation of the Amphia hospital. Statistical analyses of the CIP use data were performed using segmented regression analysis to allow for both stepwise changes and changes in trends, accounting for combined effects of interventions on both [15]. Bayesian Model Averaging (BMA) was used to account for model uncertainty by selecting the most likely models (those with the highest posterior probability) and to obtain parameter estimates averaged over the most probable models (weighting the models by posterior probability) [16]. Statistical analyses of the trend in CIP resistance in E. coli isolates was performed using segmented Poisson regression models with log link functions, adjusting for the total number of E. coli isolates tested for resistance. Models considered allowed for both stepwise changes and log-linear changes in trends, again allowing for cumulative effects of the different interventions and accounting for model uncertainty using BMA [16]. In all cases equal prior probabilities were assigned to possible models and estimated parameters were obtained by averaging over all models at least one twentieth as likely as the most likely model (though we report only the three most likely models in addition to the full model and model-averaged result). Confidence intervals were calculated using at least 100 bootstrap replicates. Analysis was performed in R 2.9 and Stata 10 [17,18].

RESULTS

Use of CIP IV

During the first 12 months, before the interventions were implemented, the monthly use of CIP was stable (mean: 561 PDD; range 333-634). During the year 2006, 181 patients on CIP IV were considered for a switch to oral. According to the protocol 136 (76 %) were suitable and in 92 (51%) patients a switch was performed.

Directly after the start of the intervention in January 2006, the IV use was reduced (Figure 1). In the segmented regression analysis of the CIP IV data, the most reliable estimate of the combined effects due to the interventions (the model averaged results) revealed strong evidence that intervention 1 was associated with a large sudden fall in IV CIP use, with a probability of 1 of a stepwise change due to this intervention. In contrast the full model (incorporating cumulative
stepwise changes in levels and changes in trends associated with each intervention) suggested that there was no evidence that any of the four interventions had any impact (Table 1).

However, because of multicolinearity these full model parameter estimates were unstable and therefore unreliable. There was considerable model uncertainty (bottom row, table 1), with the three best-fitting models all having similar posterior probabilities, close to 10%. This model uncertainty is accounted for in the model-averaged results. In the single most likely model (Model 1) IV use significantly decreased after intervention 4, but accounting for model uncertainty suggested the true effect was smaller and consistent with chance. Only the effect of intervention 1 was robust to model uncertainty. The annual use of CIP IV in the hospital decreased from 1544 PDD in 2005 to 696 PDD in 2006 (55% reduction) and with 384 PDD in 2007 (75% reduction compared to 2005).

**Total use of CIP**

The interventions targeted at the overall reduction of the use of CIP (IV and oral) started with intervention 2 in May/June 2006. Using segmented regression analysis 19 models were selected. In this case the full model, the single best model (model 1), and the model-averaged results all agreed and showed strong evidence of a step reduction in CIP use associated with intervention 2 (Figure 2, Table 2).

The best estimate of this (the model averaged result, table 2) is a reduction of 131 PDD/month (95% CI: 34, 228). The annual total use of CIP in the hospital, ICU excluded, decreased

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**Figure 1:** Monthly use of intravenous ciprofloxacin from 2005 through 2007 in PDD. Values predicted by the best segmented regression model are shown by the solid line. Broken lines show 95% confidence intervals.
Table 1: Segmented regression results for the ciprofloxacin intravenous data. Results of the model-averaged estimates, full model and three best fitting models selected by Bayesian Model Averaging are shown.

<table>
<thead>
<tr>
<th>IV(^a) CIP(^b)</th>
<th>Model averaged Coefficient(^c) (SD(^d))</th>
<th>Probability of an intervention effect(^e)</th>
<th>Full Model</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention 1</td>
<td>-67.9 (17.0)</td>
<td>1</td>
<td>-61.9 (-138.6, 14.9)</td>
<td>0.11</td>
<td>-70.7 (-94.6, -46.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intervention 2</td>
<td>-1.3 (6.6)</td>
<td>0.07</td>
<td>-30.8 (-115.5, 54.0)</td>
<td>0.46</td>
<td>-53.1 (-206.9, 100.7)</td>
<td>0.48</td>
</tr>
<tr>
<td>Intervention 3</td>
<td>-4.3 (11.5)</td>
<td>0.20</td>
<td>-24.2 (-100.5, 52.1)</td>
<td>0.52</td>
<td>-26.0 (-50.0, -2.0)</td>
<td>0.035</td>
</tr>
<tr>
<td>Intervention 4</td>
<td>-7.0 (14.6)</td>
<td>0.30</td>
<td>0.78 (-4.6, 6.2)</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trend prior to intervention change in trend</td>
<td>0.1 (0.5)</td>
<td>0.09</td>
<td>0.78 (-4.6, 6.2)</td>
<td>0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after intervention 1</td>
<td>0.4 (2.0)</td>
<td>0.16</td>
<td>-3.2 (-24.4, 18.1)</td>
<td>0.76</td>
<td>-1.8 (-3.5, -0.03)</td>
<td>0.05</td>
</tr>
<tr>
<td>after intervention 2</td>
<td>0.02 (1.9)</td>
<td>0.13</td>
<td>11.6 (17.5, 40.6)</td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after intervention 3</td>
<td>0.12 (0.9)</td>
<td>11.6 (17.5, 40.6)</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>after intervention 4</td>
<td>0.10 (0.9)</td>
<td>11.6 (17.5, 40.6)</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior model probability</td>
<td>&lt;0.01</td>
<td>0.13</td>
<td>0.11</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) IV: Intravenous
\(^b\) CIP: ciprofloxacin
\(^c\) Expected value of coefficient obtained by Bayesian Model Averaging, combining results from multiple models, weighted according to model probability (see methods)
\(^d\) SD: Standard Deviation
\(^e\) Probability coefficient is not zero in the Bayesian Model Averaging results
Improving Antimicrobial Use in the Hospital: Targeting Fluoroquinolones Using Multiple Interventions

from 6530 PDD in 2005 to 5681 PDD in 2006 (13% reduction) and to 3670 PDD in 2007 (42% reduction compared to 2005). The annual hospital use of CIP decreased from 2.7 DDD/100 patient days in 2005 to 1.7 DDD CIP/100 patient days in 2007 (Figure 3). The total antimicrobial use in the hospital increased in the years before the intervention. This increase levelled off in 2006 and 2007, as shown in Figure 3. No increase in specific groups of antimicrobial agents was observed and no new antimicrobials were used.

**Resistance rates in* E. coli*

Before the start of interventions the CIP resistance rate in* E. coli* was increasing by about 5% per year (Figure 4, Table 3). In the best fitting Poisson model for the resistance data, a significant stepwise decrease was found to be associated with interventions 2 and 4. However, there was substantial uncertainty in the model choice (the most likely model only had a posterior probability of 0.11) and after accounting for this in the model averaged results there was no conclusive evidence in support of any particular intervention. There was, however, evidence that at least one of interventions 2, 3 and 4 was associated with the observed stepwise reduction in resistance; all the best-fitting models included reductions associated with at least one of these. There was little evidence for the efficacy of intervention 1. Very strong evidence was found for an initial increasing trend, and little support for models that included a decreasing
Table 2: Segmented regression results for the total ciprofloxacin data. Results of the model-averaged estimates, full model and three best fitting models selected by BMA are shown.

<table>
<thead>
<tr>
<th>CIPa</th>
<th>Model averaged Coefficient [SD^b]</th>
<th>Probability of an intervention effect [c]</th>
<th>Full Model</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>P</td>
<td>Coefficient (95% CI)</td>
<td>P</td>
<td>Coefficient (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Intervention 1</td>
<td>0.4 (8.7)</td>
<td>0.05</td>
<td>-9.3 (-196.7, 178.1)</td>
<td>0.92</td>
<td>-107.0 (-157.6, -56.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intervention 2</td>
<td>-130.8 (49.4)</td>
<td>1</td>
<td>-249.0 (-455.9, -42.2)</td>
<td>0.02</td>
<td>-136.2 (-204.2, -68.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intervention 3</td>
<td>4.9 (19.5)</td>
<td>0.12</td>
<td>-86.8 (-462.2, 288.7)</td>
<td>0.64</td>
<td>39.6 (-30.0, 109.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>Intervention 4</td>
<td>4.7 (19.5)</td>
<td>0.11</td>
<td>18.5 (-167.8, 204.8)</td>
<td>0.84</td>
<td>-128.7 (184.2, -73.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trend prior to intervention</td>
<td>0.004 (0.9)</td>
<td>0.07</td>
<td>-5.2 (-18.5, 8.0)</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in trend</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>after intervention 1</td>
<td>0.8 (3.3)</td>
<td>0.13</td>
<td>20.3 (-31.6, 72.2)</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after intervention 2</td>
<td>1.4 (6.3)</td>
<td>0.13</td>
<td>10.7 (-60.2, 81.7)</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after intervention 3</td>
<td>-0.5 (8.3)</td>
<td>0.16</td>
<td>15.2 (-214.8, 245.1)</td>
<td>0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after intervention 4</td>
<td>-1.5 (7.7)</td>
<td>0.16</td>
<td>-44.6 (-269.4, 180.2)</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior model probability</td>
<td>&lt;0.01</td>
<td>0.29</td>
<td>0.09</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)CIP: ciprofloxacin
\(^b\)Expected value of coefficient obtained by Bayesian Model Averaging, combining results from multiple models, weighted according to model probability (see methods)
\(^c\)SD: Standard Deviation
\(^d\)Probability coefficient is not zero in the Bayesian Model Averaging results
trend term associated with interventions. Thus interventions 2-4 may have caused a stepwise reduction in resistance but were unable to reverse the increasing trend.

Resistance rates for CFRX, CFTZ, TMP-SMZ and TOBR were studied. There was strong evidence for an increasing trend in resistance to CRFX and CFTZ. None of the interventions had any effect on these resistance rates. The resistance to TMP-SMZ and TOBR showed no trend up or down and the interventions did not affect the observed resistance rates.
Table 3: Segmented Poisson regression results for the ciprofloxacin resistance data. Results of the model-averaged estimates, full model and three best fitting models selected by BMA are shown. Confidence Intervals are calculated by bootstrapping.

<table>
<thead>
<tr>
<th>CIP&lt;sup&gt;a&lt;/sup&gt; Resistance</th>
<th>Model averaged Coefficient&lt;sup&gt;b&lt;/sup&gt; (SD&lt;sup&gt;c&lt;/sup&gt;)</th>
<th>Probability of an intervention effect&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Full Model</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IRR&lt;sup&gt;e&lt;/sup&gt; (95% CI)</td>
<td>P</td>
<td>IRR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Intervention 1</td>
<td>0.98 (0.08)</td>
<td>0.12</td>
<td>0.78 (0.16, 3.82)</td>
<td>0.76</td>
<td>0.71 (0.50, 1.00)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Intervention 2</td>
<td>0.90 (0.16)</td>
<td>0.34</td>
<td>0.56 (0.22, 1.45)</td>
<td>0.23</td>
<td>0.56 (0.22, 1.45)</td>
<td>0.23</td>
</tr>
<tr>
<td>Intervention 3</td>
<td>0.77 (0.24)</td>
<td>0.52</td>
<td>0.48 (0.26, 0.89)</td>
<td>0.02</td>
<td>0.49 (0.38, 0.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intervention 4</td>
<td>0.71 (0.24)</td>
<td>0.61</td>
<td>0.57 (0.20, 1.60)</td>
<td>0.29</td>
<td>0.49 (0.38, 0.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trend prior to intervention</td>
<td>1.04 (0.01)</td>
<td>1</td>
<td>1.05 (1.03, 1.07)</td>
<td>&lt;0.001</td>
<td>1.05 (1.03, 1.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in trend after</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intervention 1</td>
<td>0.99 (0.02)</td>
<td>0.19</td>
<td>1.04 (0.68, 1.58)</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intervention 2</td>
<td>1.00 (0.02)</td>
<td>0.13</td>
<td>1.05 (0.69, 1.60)</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intervention 3</td>
<td>0.99 (0.07)</td>
<td>0.15</td>
<td>1.10 (0.53, 2.31)</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intervention 4</td>
<td>1.02 (0.07)</td>
<td>0.15</td>
<td>0.83 (0.41, 1.67)</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>CIP: ciprofloxacin  
<sup>b</sup>Expected value of coefficient obtained by Bayesian Model Averaging, combining results from multiple models, weighted according to model probability (see methods)  
<sup>c</sup>SD: Standard Deviation  
<sup>d</sup>Probability coefficient is not zero in the Bayesian Model Averaging results.  
<sup>e</sup>IRR: Incidence Rate Ratio
Costs and savings

Considering only the price of the agent, switching patients from IV CIP to oral CIP saved the hospital about €114,000 over 2 years, based on the CIP price in 2005. Overall savings were higher than this but the additional savings were not calculated because reliable data were not available. The annual costs of the program were approximately €32,000 based on the salary of the study coordinator and the pharmacy assistant.

DISCUSSION

This study shows that implementation of a bundle of interventions targeted at the improving use of CIP were associated with a significant decrease in use of IV CIP as well as a decrease of the overall use of CIP. The IV use of CIP decreased immediately after the first intervention (the SWITCH project). The SWITCH project improved the quality of care and resulted in important savings, which were sufficient to fund the entire project. The IV-to-oral switch had several other advantages, which include decreasing the risk of complications from IV-catheters, increasing the patients comfort and mobility, and discharging the patient from the hospital earlier because the patient no longer has an IV catheter [8,19].

The overall use of CIP was reduced which was most likely caused by intervention 2, the introduction of a new guideline and an educational program. We did not detect an additional effect of interventions 3 and 4, but we cannot determine if these interventions influenced the sustainability of the effect. Therefore, we conclude that the combination of interventions reduced the overall use of CIP and that intervention two was an essential part of the bundle. Intervention two was relatively simple and required limited resources. Van Hees et al. described a similar significant reduction due to educational interventions targeted at CIP use, in a hospital with a low rate of CIP use (5.7 DDD/100 patient days in 2004) [20]. However, the CIP use data were difficult to compare with our findings because the antimicrobial use was expressed in number of prescriptions per 1000 admissions and only a limited number of wards were included in the intervention program. These studies show the effect of having antimicrobial guidelines and implementing them locally by using educational sessions on the antimicrobial use.

To put these findings into perspective it is important to understand that the current study was performed in a setting with a relatively low fluoroquinolone use, also compared with most Dutch hospitals [4]. The average Dutch hospital use of fluoroquinolones in 2005 was 7.4 DDD/100 patient days [21]. This is nearly three times higher than the use in our hospital in 2005 (Figure 3). During the study period, use of CIP declined further to 1.7 DDD/100 patient days in 2007. At the same time the use in other Dutch hospitals further increased [21].
Although it is generally agreed upon that more antimicrobial use results in an increase of resistance, it is unclear if the opposite is also true. We measured the susceptibility of E. coli since this organism is an important target for fluoroquinolones and it is the most frequently encountered clinically relevant species of the Enterobacteriaceae.

Before the start of the interventions the observed resistance of CIP increased 4.6% annually (figure 4). This increase was interrupted by a stepwise decrease in resistance and this was most likely associated with interventions 2 and 4. The data showed that at least one intervention was associated with a reduction in resistance, but there was no conclusive evidence to determine which intervention.

There are several difficulties in determining the association of the interventions with the observed resistance. The relationship between the amount of antimicrobial use and the development of resistance has been clearly established in both the community [10,22-24] and in the hospital setting [25,26]. However, the extent to which a trend toward increasing resistance can be reversed by changes in prescribing is less clear.

For example, complete cessation of sulfonamide use in the UK did not lead to a decrease of resistance in E. coli during the 1990s [27]. A likely explanation is that plasmids containing sulfonamide-resistance determinants also contained genes encoding resistance to other antibiotics and that continued use of these agents during the study period maintained the selective pressure for the multi-resistant plasmids [27,28]. Lipsitch concluded that interventions to control antimicrobial use could decrease resistance, but expectations for their success should be moderate as the relations are indirect and non-linear [28]. He postulated that, in the community, in the most successful cases, five years or more are required to observe a substantial decline in resistance [29]. Van Eldere et al also reported that changes in observed resistance among Streptococcus pneumoniae was delayed after usage of broad-spectrum penicillins decreased [30].

Of note, this study described a reservoir of resistance in the community which is probably different from that in the hospital setting. In our hospital-based study, the change in resistance rate occurred approximately 6 months after the use was reduced. In contrast to the community, changes in antimicrobial prescription in hospitals could have a much more rapid effect, because of the ‘dilution’ effect of the newly admitted patients [28]. This hypothesis is based on the assumption that cross infection with resistant strains is minimal and that resistance rates among out-patients are lower than among the hospitalised patients.

Recent studies in our hospital found a low rate of nosocomial transmission of highly resistant Gram-negative bacteria. The number of infections due to a strain and acquired by nosocomial transmission divided by the number of infections due to strains of the same species and not acquired by nosocomial transmission was 0.05 [31]. Also, the resistance rate for CIP in the community in the Netherlands is relatively low. A recent study reported 3% CIP resistance among bacterial pathogens isolated from patients on admission to the hospital [32]. Moreover, we previously demonstrated that CIP resistance on individual medical wards correlated with the
amount of use on the individual wards [26]. These data suggests that the volume of antimi-
crobial use in the hospital is an important determinant for the observed resistance. Our study
design prevents us from making strong statements what caused the decrease in CIP resistance.
We conclude that it is at least plausible that the decreased incidence of CIP resistant *E. coli* iso-
lates, cultured from clinical patients, resulted from changes in the levels of fluoroquinolone use.

There are some limitations of this study: First, we used a quasi experimental interrupted time
series design which has well-known limitations [33]. Some of these we were able to overcome
through use of a segmented regression analysis, the most appropriate method for assessing
the effects of our interventions to reduce use of CIP and other antimicrobial agents. However,
it is less clear how we should analyse and interpret the subsequent effects on observed
resistance given the uncertainties about the mechanisms selected for the resistant micro-
organisms[28,29].

Second, the ICU was excluded from the study. We did not include that unit because the
consultant microbiologists, involved in the study, visited the ICU on a daily basis to advise
physicians about antimicrobial treatment. Therefore, we assumed that we would not be able to
improve antimicrobial use in this unit.

Third, the follow-up period was relatively short, which prevented us from determining the
long-term effect of this intervention on antimicrobial resistance.

The observed effect on the CIP resistance rates could also be biased by the occurrence of
outbreaks or by changes in the infection control policy that were not part of the bundle. As
mentioned before, we measured the occurrence of horizontal spread of resistant micro-organ-
isms in the hospital during the study period and found a very low rate of transmission and no
major outbreaks were observed [31]. The infection control policy regarding the prevention of
transmission of resistant micro-organisms was implemented before 2005 and no changes were
made since then. Therefore, it is unlikely that the observed resistance rates were influenced by
these factors.

The cost analysis was limited to the change from IV to oral CIP. This was a very clear intervention
with no hidden costs or substitution effects. Effects of the reduced total use of CIP were not
included in the cost-analysis. Although there are likely cost-savings associated with this as well,
these are much more difficult to quantify. The total amount of antibiotic use did not increase
and no new, more expensive, agents were used during the study period. Therefore, the savings
calculated in this manuscript are a minimal estimate. Still, this minimal estimate was sufficient
to pay for the costs of this project.

In conclusion, multiple targeted interventions improved the use of CIP in our hospital. A SWITCH
project from IV to oral was successful and saved money. Subsequent interventions including
the introduction of a new guideline and an educational program reduced overall CIP use by
30%. An increasing trend in the observed resistance among *E. coli* was reversed in association with the decrease in CIP use. These findings show that a bundle of interventions can reduce the use of antimicrobial agents in a hospital and could reverse the increase in antimicrobial resistance.

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GENERAL DISCUSSION

Initially, antimicrobial control policies were initiated mainly to control costs and not to limit the development of resistance [1-3]. Nowadays, the opposite is true, the costs of most antimicrobial agents being extremely low, and the selective pressure of antimicrobial therapy (AMT) resulting in increased resistance concerns the whole medical world. [4,5].

The purpose of the studies presented in this thesis was to gain more insight into the prescription of AMT and the epidemiology of resistant micro-organisms in hospitals. Based on these findings a quality improvement program was implemented.

Monitoring of antimicrobial use in hospitalised patients

The most common method to monitor the use of AMT is based on consumption data, expressed as Defined Daily Doses (DDD) per patient day [6]. Filius et al. adapted this method and concluded that DDD per 100 admissions was an additional measurement, which was especially useful to compare antimicrobial consumption rates over time or between different hospitals, geographical regions or countries, because it was less sensitive to changes in hospital resource indicators [7]. These methods provide information about the quantitative use of antimicrobials, however no information about the appropriateness of use is provided.

We developed a point prevalence survey on antimicrobial use in which the appropriateness of use of AMT can be judged on a patient level. In addition, demographic and infection-related data can be collected to study potential determinants of inappropriate use of AMT. Determinants of inappropriate use of AMT can subsequently be used to develop targeted interventions. By repeating the prevalence surveys, the effectiveness of interventions can be measured. The repeated prevalence surveys in combination with targeted interventions results in a true quality circle for AMT in hospitals.

There are three major requirements for the successful implementation of a prevalence survey of AMT. First, there have to be clear and explicit antimicrobial prescription guidelines in the hospital. A second requirement is the knowledge and decisiveness of the persons who are judging the therapy; and finally, the availability of and access to medical records (chapter 2.2). The assessment of appropriateness of AMT on a patient level can be very complicated. It is therefore important that the local guidelines are explicit in their choices. These guidelines should be used strictly when judging the appropriateness.

In chapter 2.1 it is shown that the prevalence surveys were successfully performed in our hospital, with a very low proportion of cases in which no judgment could be made. However, when this method was implemented in other hospitals, many hospitals had a relatively high percentage of cases in which no judgment could be given (chapter 2.2). More intensive training is necessary to implement this method in other hospitals.

The prevalence surveys described in this thesis (chapter 2.1 and 2.2) were performed in a country with an extremely low use of AMT [8]. Nevertheless, a substantial proportion of
the patients on AMT did not require AMT. The conclusion is that there is enough room for improvement of AMT and that prevalence surveys are useful tools to measure the amount and appropriateness of AMT.

**Incidence and transmission of Highly Resistant Micro-organisms (HRMO)**

The original concept of antimicrobial resistance was that resistance was a result of spontaneous mutation and subsequent selection by antimicrobial use [9]. The widespread development of multi-drug resistance in many species of bacteria led scientists to believe that other mechanisms presumably play a role as well. Horizontal gene transfer is a process whereby genetic material contained in small packets of DNA can be transferred between individual bacteria [10]. Nowadays this is considered the most important mechanism for acquired resistance in clinically relevant species. In general antimicrobial use does not actively induce resistance. It provides a selective advantage of resistant strains by suppressing the normal flora [11,12]. Some antimicrobial agents, e.g. fluoroquinolones may play a more active role by stimulating gene transfer [13,14].

A restrictive antibiotic policy is considered essential to maintain low resistance rates. Another factor essential to limit the rate of transmission is an active infection control program. This consists mainly of good hand hygiene compliance, isolation of patients infected with resistant bacteria, screening high risk patients and contact tracing in case of unexpected findings.

Theoretically the incidence of Highly Resistant Micro-organisms (HRMO) in hospitals is influenced by the use of AMT and by the infection control policy. A high incidence is most likely when the use of AMT is high and infection control is poor. The effects of these measures have hardly been studied in clinical practice.

**Surveillance of Highly Resistant Micro-organisms (HRMO)**

A clear definition for resistance is required to determine the incidence of HRMO. Most studies mention the percentage of resistant strains against individual antimicrobial agents [15,16]. However, multidrug resistance is the true problem that hampers clinical management of patients and this has to be taken into account as well. It is almost impossible to compare incidences of HRMO, because of the variation in definitions used. One study used the criterion of being susceptible to less than two groups of bactericide antibiotics [17] and one defined “pan-resistance (i.e. decreased susceptibility to cefepime, ceftazidime, imipenem, meropenem, piperacillin/tazobactam, ciproflocacin and levofloxacin)” [18].

In the past years the international medical community (CDC, IDSA, ECDC and WHO), has attempted to highlight the antimicrobial-resistance problem, including the need for accurate surveillance. Nevertheless a general agreement about a universal definition of multi-drug resistant has not been obtained [16,19-21]. The definition used in the National Dutch guideline is based on both single-drug resistance and multidrug resistance, depending on the impact for individual patients and clinical decisions [22,23]. This definition covers the entire spectrum of clinically relevant bacteria (excluding Mycobacterium species).
This definition was used in the surveillance studies in chapter 3. In a single-centre study, performed in a large teaching hospital (chapter 3.2), we determined an incidence density of 43 HRMO / 100,000 patient days, this included Highly Resistant – Gram negative Rods (HR-GNR), Penicillin Non-susceptible S. pneumoniae (PNSP) and Methicillin-Resistant S. aureus (MRSA). The majority were HR-GNR, with an incidence density of 35/100,000 patient days.

Molecular typing of the strains was used to investigate the genetic relatedness of HRMO. Analyses of epidemiologically linkage within the window period indicated that the rate of transmission for HR-GNR and PNSP was relatively low (Transmission Index of 0.05 and 0.29 respectively). Transmission of MRSA was not observed. Transmission of PNSP always occurred when the patient had not been placed in isolation, because laboratory results were not available yet. Once droplet precautions had been installed no further transmission was observed. This illustrates the need for more rapid laboratory detection of resistant isolates.

In HR-GNR, besides the transmission of the bacterium (HR-GNR) itself, the transmission of transmissible plasmids and integrons may also play a role. In the study, described in 3.3, the role of integron transmission was investigated. The transmission index of integrons was nearly twice the transmission of the HR-GNR themselves. This shows that, at least in this hospital, the contribution of horizontal gene transfer to the spread of resistance is greater than that of horizontal spread of bacteria. Only a few studies have investigated the role of mobile genetic elements in the population dynamics of bacterial resistance. The results of this study show that more attention should be directed towards the role of mobile genetic elements.

To obtain more insight in the occurrence of HRMO, and especially HR-GNR, and their transmission in Dutch hospitals a multi-centre study was initiated, the TRIANGLE study (chapter 3.4). The design was based on the single-centre study, described in chapter 3.2. All hospitals, university-, teaching- and non-teaching hospitals, used the same set of definitions for HR-GNR, as defined in the Dutch national Guideline [22].

The mean observed incidence density of HR-GNR was 43/100,000 patient days and a large range was observed in the 18 hospitals that participated (range 8 – 123 per 100,000 patient days; mean 43; SD 38). The only independent determinant associated with the incidence of HR-GNR was staying in a university hospital, which is considered to be a surrogate marker for the complexity of the patient population and the complexity of care provided.

In the general hospitals no transmission of HR-GNR was observed. The adjusted Transmission Index (adjusted for the proportion of available HR-GNR isolates) in the other hospitals ranged from 0.0 through 0.2 (mean 0.06; SD 0.05). No independent determinants associated with a higher transmission were identified.

In both the single- and the multicentre study, described in chapter 3.2 and 3.4 we proved that nosocomial transmission of HR-GNR was effectively controlled in all hospitals using trans-
mission-based precautions. Although the transmission index varied considerably between the participating hospitals, it was always far below the epidemic level (≥1.0).

The proportion of HR-GNR that was caused by nosocomial transmission of bacteria was only a minority of all HR-GNR found (chapter 3.2 and 3.4). Therefore the main source of HR-GNR was likely to be the endogenous flora of the patient already present on admission to the hospital [24,25]. This implicates that there probably are community reservoirs of resistance that have to be identified to remain in control of antimicrobial resistance in the future.

**Focussing on fluoroquinolone use**

The prevalence survey in chapter 2.1 identified the use of fluoroquinolones as an independent determinant of inappropriate AMT. In chapter 3.1, it is shown that fluoroquinolones are associated with a more rapid development of resistance than beta-lactam antibiotics. Based on these results, it is concluded that fluoroquinolones are more prone to induce resistance in microorganisms than beta-lactams. In 1987, Philips et al. already reported that the use of fluoroquinolones promotes horizontal dissemination of antimicrobial resistance genes by activating an SOS response, which was later confirmed by Beaber et al. [13,14]. This may explain why the use of fluoroquinolones results in an extremely rapid dissemination of resistance genes. This is worrying, considering the increase of fluoroquinolone use and the large proportion of inappropriate use of fluoroquinolones (chapter 2.1 and 2.2). In 1999, only 11 years ago, Thomson reported on the global epidemiology of resistance. He concluded that the results of sensitivity testing to ciprofloxacin were encouraging and he challenged the users to minimise the use [24]. Today, the highest amount of fluoroquinolone use in Europe in outpatients is found in Greece (around 1.0 DDD/100 inhabitants/day in 2004). This is about 10 times more than in the Netherlands (0.1 DDD/100 inhabitants/day in 2008) [8,27]. Fluoroquinolone resistance was found in 64% of all strains of Klebsiella pneumoniae isolated from blood cultures in Greece. In the Netherlands only 7% of all K. pneumoniae strains isolated from blood cultures were fluoroquinolone resistant in 2008 [28].

Although the use of fluoroquinolones in The Netherlands is relatively low, we identified fluoroquinolones as the most important determinant of inappropriate use of AMT. The use of fluoroquinolones is increasing and the resistance rates are going up. In the HR-GNR collection, obtained during the TRIANGLE study, more than half of the Enterobacteriaceae were resistant to ciprofloxacin (chapter 3.4). Considering these findings an intervention was started to reduce the use of fluoroquinolones.

**Improving antimicrobial use by interventions**

The success of interventions to improve AMT depends on several parameters. Firstly, the support from the hospital director or management before starting an intervention. The intervention studies in chapter 4 were financially supported by the hospital. A business case was made predicting that the standardisation of the protocol for peri-operative prophylaxis in
surgical procedures and the ciprofloxacin SWITCH project would result in cost savings that were sufficient to fund the personnel costs for all projects in this thesis. These assumptions were confirmed afterwards.

A second essential factor for a successful intervention is a dedicated project-coordinator, who coordinates a multidisciplinary team (i.e. Microbiologist, Pharmacist, Physicians) [29,30].

Thirdly, the intervention protocols and the antimicrobial prescription guidelines should be as straightforward as possible. Both the content and the implementation in the clinical setting should be simple. In the study described in chapter 4.1, it was likely that the simplicity of the guideline, in combination with personal instruction, were critical success factors for the implementation and the improvement on timing of prophylaxis. In the ciprofloxacin interventions (chapter 4.2), again, it was the simplicity of the procedure (SWITCH according to 3 criteria) and the personal instruction (one-on-one education & feedback strategy), which made the intervention successful. Personal approach with feedback is the fourth factor that improves the outcome of an intervention [31,32]. Finally, education is an important aspect and this should be targeted at learning needs and include interactive educational activities. [33]. All these conditions were met in the study described in chapter 4.2.

The effects of the interventions can be measured using a quasi-experimental design (before-after measurement), as performed in the perioperative antimicrobial prophylaxis intervention (chapter 4.1). Use of this methodology might overestimate or underestimate the effect of an intervention because natural trends are not taken into consideration. A study design using interrupted time series with segmented regression allows for both stepwise changes and changes in trends (used in the ciprofloxacin intervention study in chapter 4.2) [34-36]. This is a useful method to measure the effect of the interventions on the use of AMT.

The intravenous use of ciprofloxacin was reduced with 71 prescribed daily doses (PDD) per month (95% CI: 47-97) in association with a SWITCH intervention and the total use of ciprofloxacin (iv and oral) was reduced with 107 PDD per month (95% CI: 56-158) using a bundle of interventions. The interventions that were most likely to have had an effect on the overall use were the introduction of a new antimicrobial guideline and an educational program. Although there was a clear effect on the amount of use, the effect on the observed resistance rates is more complex as the relations are probably non-linear or indirect [37]. Nevertheless, the model indicated that the reduced use of fluoroquinolones had a significant effect on the increasing trend of fluoroquinolone resistance.

Before the start of the bundle of interventions the ciprofloxacin resistance rate in *E. coli* was increasing with 4.6% per year. The statistical model revealed a significant stepwise decrease associated with the bundle of interventions on ciprofloxacin use. This is one of the few studies that found an effect of reduced use of AMT on resistance rates. Considering the uncertainty whether reduced use can lower resistance rates once these are established the conclusion must be that it is of utmost importance to prevent the development of resistance at a very early stage.
CONCLUSIONS

In conclusion, the prevalence surveys described in this thesis are useful methods to measure the amount and the appropriateness of AMT. Determinants of inappropriate use were identified and served as targets for interventions. The effect of the interventions can be measured by ongoing prevalence surveys.

Surveillance of HRMO in a teaching hospital revealed that the Intensive Care Unit was a main determinant for the presence of HRMO, and that the transmission of HRMO could be largely prevented using transmission precautions. A multi-centre study, based on the same method, was performed to determine the incidence and transmission of HR-GNR and the relation with infection control precautions, type of hospital (university, teaching, non-teaching) and antimicrobial use. It showed that there was considerable variation of the incidence density that was related to the complexity of the patient population and of the care provided. Nosocomial transmission of HR-GNR was effectively controlled in all hospitals and played a minor role. Additional research on the presence of integrons showed that the contribution of horizontal gene transfer to the spread of resistance is larger than that of nosocomial transmission of bacteria.

Finally, a bundle of interventions that was implemented to improve the use of fluoroquinolones resulted in substantial cost-savings as well as a marked reduction in the hospital use of fluoroquinolones. Furthermore, the reduction in fluoroquinolone use was associated with a change in the increasing trend of the observed resistance against ciprofloxacin.

The studies in this thesis provide information on the relation between the use of antimicrobials, infection control and antimicrobial resistance in hospitals. They can be used as a benchmark for others and to monitor future trends. It was shown that even in a setting with a relatively low use of antimicrobial agents, substantial improvements can be achieved.

Considerations for the future

In Europe, the ECDC published a report called “The bacterial challenge: time to react”, in which the yearly burden of infections due to selected antibiotic-resistant bacteria were estimated [16]. The estimated number of additional deaths and hospital days, due to bloodstream infections caused by third-generation cephalosporin-resistant E. coli and third-generation cephalosporin-resistant Klebsiella spp., was 8,000 deaths and 566,000 hospital days (EU Member States, Iceland and Norway in 2007).

During the TRIANGLe study period, 70 highly resistant E. coli and Klebsiella spp. were recovered from blood cultures. Using the Dutch hospital resource indicators, obtained from the Dutch Hospital Data information office, the TRIANGLe findings can be converted to a national figure for the Netherlands of 605 bloodstream infections with highly resistant E. coli and Klebsiella spp. [38]. Using the estimates for attributable mortality and costs from the ECDC, this result in 94 additional deaths and 6,674 additional hospital days. Compared to the total additional deaths and hospital days in Europe, the Dutch figure is just over one percent. This figure might
be an overestimation because of the large contribution of university hospitals in this study. However, in the Netherlands, the incidence of HR-GNR is increasing rapidly, and so will the hospital costs and the number of deaths due to an infection with a resistant micro-organism [27].

We can conclude that the guidelines for infection control and the restrictive antimicrobial policy in the Netherlands are effective. To maintain this situation, the current policy should be continued but additional measures are needed to remain in control. New research has to be initiated to define sources of resistant micro-organisms outside the hospital, to elucidate the transmission dynamics of mobile genetic resistance elements and to investigate new resistance mechanisms, like metallo carbapenemases. Our results can be used to optimise current control strategies and to monitor the development of resistance in the future.
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Samenvatting en Conclusies
SAMENVATTING

Antibiotica zijn belangrijke geneesmiddelen die zorgvuldig gebruikt moeten worden. Aanvankelijk werd uitsluitend op het gebruik van antimicrobiële middelen gelet om kosten te beheersen en niet om de ontwikkeling van resistentie te beperken. Tegenwoordig geldt het tegendeel, de kosten van de meeste antimicrobiële middelen zijn laag, en de selectieve druk van antimicrobiële therapie (AMT) leidt, wereldwijd, tot een enorme toename van resistentie.

De in dit proefschrift beschreven studies zijn opgezet om meer inzicht te krijgen in het gebruik van AMT en de epidemiologie van resisteante micro-organismen in de Nederlandse ziekenhuizen. Op basis van deze bevindingen zijn diverse kwaliteitverbeteringsprogramma’s geïnitieerd.

Het is belangrijk om naast het kwantitatieve gebruik van AMT ook inzicht te hebben in het kwalitatieve gebruik. Dit proefschrift beschrijft, in hoofdstuk 2, een methode om de kwaliteit te meten door het uitvoeren van één of meerdere punt prevalentie onderzoek(en). Hierdoor is het mogelijk om op individueel patiënt niveau inzicht te krijgen in de juistheid van het gebruik van AMT. Tevens is het mogelijk om determinanten, die geassocieerd zijn met onjuist gebruik, te determineren. Deze determinanten kunnen vervolgens worden gebruikt om doelgericht verbeteringsprogramma’s uit te voeren.

Het prevalentie onderzoek, beschreven in dit proefschrift, is uitgevoerd in een land met een relatief laag gebruik van AMT. Toch bleek een substantieel deel van de patiënten onnodig AMT te krijgen. Hieruit blijkt dat er nog steeds voldoende ruimte is voor verbetering.

Een terughoudend antibioticabeleid is essentieel om de incidentie van bijzonder resisteante micro-organismen (BRMO) laag te houden omdat door het veelvuldig en onjuist gebruik van antimicrobiële middelen een milieu gecreëerd wordt waarin BRMO in het voordeel zijn. Daarnaast is het van belang om de transmissie van de BRMO tegen te gaan door middel van een actief infectiepreventiebeleid. Dit bestaat onder andere uit isolatie van gekoloniseerde patiënten, screening van hoog-risico patiënten en het uitvoeren van een contactonderzoek na een onverwachte bevinding van een BRMO in het ziekenhuis. De relatieve bijdrage van het antibioticabeleid en de infectiepreventie maatregelen ten aanzien van de incidentie en transmissie van BRMO zijn nog niet eerder onderzocht tijdens een endemische situatie.

Hoofdstuk 3 beschrijft twee studies waarin de incidentie en transmissie van BRMO is onderzocht. Alvorens te kunnen beginnen met dergelijk onderzoek moeten eenduidige definities worden opgesteld van BRMO. Wereldwijd worden diverse definities gebruikt waardoor vergelijken van onderzoeksresultaten bijna onmogelijk is. Een universele definitie van BRMO is daarom een vereiste voor de toekomst.

De in hoofdstuk 3 gebruikte definitie van BRMO uit de nationale Nederlandse richtlijn is gebaseerd op zowel resistentie voor enkelvoudige antibiotica als resistentie tegen een
combinatie van antibiotica en is vooral afhankelijk van de impact voor de individuele patiënt en de klinische besluitvorming voor behandeling.

Eerst werd in één centrum de incidentie van BRMO gemeten. Deze was 43 BRMO per 100.000 patiëntdagen, waarvan 35 bijzonder resisteante Gram-negatieve staven (BRGNS) per 100.000 patiëntdagen. De afdeling intensieve zorg bleek geassocieerd te zijn met een hogere incidentie dan de rest van het ziekenhuis. De mate van transmissie is uitgedrukt in een transmissie index (ratio secundaire patiënten op primaire patiënten) en bedroeg respectievelijk 0,05 en 0,29 voor BRGNS en Penicilline ongevoelige *Streptococcus pneumoniae*. Methicilline resistente *Staphylococcus aureus* werd in geen enkel geval overgedragen naar andere patiënten.

Naast de overdracht van de bacterie zelf, kan ook de overdracht van plasmiden en integronen een rol spelen. In de studie, beschreven in hoofdstuk 3.3, is de rol van integron transmissie onderzocht. De transmissie index voor integronen was bijna twee maal zo hoog als de transmissie index voor BRGNS zelf. Dit toont aan dat de bijdrage van horizontale genoverdracht aan de totale verspreiding van resistentie groter is dan die van de horizontale verspreiding van bacteriën. De resultaten van deze studie tonen aan dat er meer aandacht moet worden gegeven aan de rol van mobiele genetische elementen in relatie tot de epidemiologie van resistentie in het ziekenhuis.

Vervolgens werd een soortgelijk onderzoek uitgevoerd in meerdere ziekenhuizen. Er werd een grote variatie in de incidentie van BRGNS gevonden (8 – 123 / 100.000 patiëntdagen). De universitaire ziekenhuizen waren de enige onafhankelijk determinant die geassocieerd was met een hogere incidentie. Dit kan beschouwd worden als een surrogaat marker voor de complexiteit van de patiëntpopulatie en van de benodigde zorg.

Nosocomiale transmissie kwam sporadisch voor en was niet geassocieerd met een hogere incidentie. Ook werden geen determinanten geïdentificeerd die correleerden met de hoogte van de transmissie index per ziekenhuis. In alle ziekenhuizen was de transmissie index ruim onder de waarde die leidt tot een epidemische verheffing.

Het aandeel van de BRGNS dat werd veroorzaakt door nosocomiale transmissie was slechts een minderheid van alle BRGNS die werden gevonden (hoofdstuk 3.2 en 3.4). Daarom is de belangrijkste bron van BRGNS zeer waarschijnlijk de endogene flora van de patiënt, die al aanwezig is bij opname in het ziekenhuis. Dit impliceert dat onderzoek naar een mogelijk reservoir van resistentie buiten het ziekenhuis meer aandacht verdient.

In hoofdstuk 3.1 staat beschreven dat het gebruik van fluoroquinolonen geassocieerd is met een snellere ontwikkeling van resistentie dan het gebruik van β-lactam antibiotica. Al in 1987 is beschreven dat fluoroquinolonen een 'SOS respons' stimuleren binnen de bacterie waardoor resistentie makkelijker ontstaat. Dit is verontrustend gezien de grote hoeveelheid fluoroquinolonen die onjuist en onnodig gebruikt wordt (hoofdstuk 2.1 en 2.2). Fluoroquinolonen waren
de enige onafhankelijke determinant van onjuist antibiotica gebruik. Dit was de aanleiding voor het starten van een verbeterprogramma gericht op het gebruik van fluoroquinolonen.

Dit proefschrift beschrijft de succesfactoren voor het laten slagen van een verbeterprogramma gericht op het verbeteren van antibiotica gebruik, zoals (1) steun van het ziekenhuisbestuur, (2) een toegewijde projectcoördinator binnen een multidisciplinair team, (3) eenvoudige en eenduidige protocollen, (4) persoonlijk benadering met feedback, (5) scholing die is aangepast aan de specifieke behoeften van de doelgroep.

Daarnaast dient vooraf te worden nagedacht over de studieopzet om het effect van het verbeterprogramma te kunnen interpreteren. Hoofdstuk 4.1 beschrijft een voor- en nameting, terwijl in hoofdstuk 4.2 een interrupted time series analyse wordt beschreven. In de laatste kunnen zowel stapsgewijze veranderingen als veranderingen in trends worden gerelateerd aan specifieke interventies.

De interventies gericht op het ciprofloxacine gebruik in het studieziekenhuis (ciprofloxacine werd het enige fluoroquinolon dat werd gebruikt) resulteerden in een reductie van het intraveneus gebruik met 71 Prescribed Daily Doses (PDD) per maand en het totale ciprofloxacine gebruik daalde met 107 PDD per maand.

De introductie van een nieuwe richtlijn voor AMT en een scholingsprogramma waren geassocieerd met de afname in gebruik. Hoewel er een duidelijk effect van de interventies op het gebruik van ciprofloxacine is gevonden, is het effect op de waargenomen resistentie minder eenduidig. Dit is, in het algemeen, moeilijker aan te tonen omdat relaties niet-lineair en indirect zijn. Niettemin is aangetoond dat de verminderende effecten van het gebruik van ciprofloxacine een significant effect had op de toenemende trend van ciprofloxacine resistentie in E. coli gekweekt bij klinische patiënten. Dit is een van de weinige studies die een effect heeft kunnen aantonen van een vermindering van het antibioticagebruik op de waargenomen resistentie. De algemene les is dan ook dat voorkomen beter is dan genezen.

Conclusies

De prevalentie onderzoeken beschreven in dit proefschrift bleken bruikbare methoden om de kwaliteit van gebruik van antimicrobiële middelen te meten. Determinanten van onjuist gebruik werden aangetoond en dienden als doelen voor gerichte verbeterprogramma’s.

Surveillance van BRGNS toonde aan dat de afdeling intensieve zorg een belangrijke determinant voor de aanwezigheid van BRGNS was. In een onderzoek over meerdere Nederlandse ziekenhuizen werd een grote variatie in de incidentie van BRGNS aangetoond, die gerelateerd was met het type ziekenhuis (universitaire ziekenhuizen).

Nosocomiale transmissie van bacteriën was onder controle in alle deelnemende ziekenhuizen en speelde een minimale rol ten opzichte van de totale hoeveelheid BRGNS. De belangrijkste bron van BRGNS was zeer waarschijnlijk de endogene flora van de patiënt.
Tot slot werd een bundel van interventies geïmplementeerd om het fluoroquinolon gebruik te verbeteren. Dit resulteerde zowel in een reductie van het gebruik van ciprofloxacin als in een trendbreuk in de stijgende ciprofloxacin resistentie bij *E. coli* gekweekt bij klinische patiënten.

De studies tonen aan dat de richtlijnen voor infectiepreventie en het restrictieve beleid ten aanzien van antimicrobiële middelen in Nederland effectief zijn. Gezien de toename van resistentie buiten het ziekenhuis zijn in de toekomst aanvullende maatregelen nodig. Meer onderzoek is nodig om de diagnostiek van resistentie micro-organismen te verbeteren en te versnellen, de epidemiologie van mobiele genetische elementen te verhelderen, de bronnen van resistentie micro-organismen buiten het ziekenhuis te identificeren en nieuwe resistentie mechanismen, zoals metallo-beta-lactamases, tijdig te herkennen en te bestrijden. Alleen dan zullen we ook in de toekomst in staat zijn om patiënten met ernstige infecties effectief te behandelen.
Dankwoord
DANKWOORD

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Na haar promotie zal zij werkzaam blijven op de afdeling infectiepreventie van het LMI.
List of Publications
LIST OF PUBLICATIONS

Improving Quinolone Use in the Hospital Using a Bundle of Interventions in an Interrupted Time Series Analysis.
Willemsen I, Cooper B, Buiten en van C, Winters M, Andriesse G, Kluytmans J.
Antimicrob Agents Chemother. 2010 Accepted

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